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DEVELOPMENT AND TRANSMISSION OF *PROTOSTRONGYLUS BOUGHTONI* (NEMATODA:METASTRONGYLOIDEA), A LUNGWORM OF THE SNOWSHOE HARE (*LEPUS AMERICANUS*)

by

(C)

ROBERT A. KRALKA

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SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

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W. M. Samuel
Supervisor

M. R. T. Dale

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Date. April 21, 1983

Abstract

The life cycle of *Protostrongylus boughtoni* Goble and Dougherty, 1943, was studied experimentally in intermediate and definitive hosts. Levels of infection and patterns of transmission of the parasite were examined in natural populations of snails and snowshoe hares (*Lepus americanus*).

In laboratory experiments, first-stage larvae of *P. boughtoni*, ingested by the snail *Vallonia pulchella*, developed in the foot to the third-stage in 28-30 days at 18°C. Some third-stage larvae emerged from the foot of infected snails. First-, second-, third-, and fourth-stage larvae of *P. boughtoni* were described.

Laboratory-reared snowshoe hares, each given 50 third-stage larvae of *P. boughtoni*, developed patent infections in 25 to 27 days post-exposure. Periods of patency ranged from 41 to 104 days.

Natural *P. boughtoni* infections in intermediate and definitive hosts were examined on a study area near Slave Lake, Alberta in 1981. Thirteen species of terrestrial gastropods were collected, and *Vertigo gouldi*, *Vertigo ovata*, *Columella edentula* and *Euconulus fulvus* were infected with *P. boughtoni* larvae. *V. gouldi*, which had the highest prevalence and intensity and was the most abundant intermediate host on the study area, was studied in detail.

Prevalence and intensity of *P. boughtoni* in *V. gouldi* were stable temporally and between habitat types. Abundance of *V. gouldi* differed between habitat types and was greatest

in dry coniferous woods. There was no relationship between size class of *V. gouldi* and prevalence or intensity of infection.

Prevalence of *P. boughtoni* in adult snowshoe hares was 100%; mean intensity varied monthly at relatively low levels. Juvenile snowshoe hares became infected within a month of birth; intensities increased to relatively high levels within three months and then declined, indicating development of an immune response. No evidence was found for transplacental transmission of *P. boughtoni*.

Relative rates of flow of *P. boughtoni* were calculated to illustrate the importance of snail species, hare age groups and habitat types in transmission of the parasite. *V. gouldi* accounted for nearly all flow from snails to hares, juvenile hares accounted for most flow from hares to snails, and the majority of flow from snails to hares occurred in dry coniferous and mixed wood habitats.

Stability of *P. boughtoni* transmission in cyclical snowshoe hare populations was discussed.

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GENERAL INTRODUCTION

Nematodes of the superfamily Metastrongyloidea, family Protostrongylidae have been the subject of much recent research because of their debilitating effects in the lungs (Pybus 1983), central nervous system (Anderson 1971) and musculature (Pybus 1983) of ruminant hosts. Well known examples of such wild-mammal pathogens are *Elaphostrongylus cervi* in caribou (*Rangifer tarandus*) (Lankester and Northcott 1979), *Parelaphostrongylus tenuis* in moose (*Alces alces*) (Anderson 1971), and *Protostrongylus stilesi* and/or *Protostrongylus rushi* in bighorn sheep (*Ovis canadensis*) (Spraker 1979). Important protostrongylid parasites of domestic sheep and goats include *Muellerius capillaris*, *Protostrongylus rufescens* and *Cystocaulus ocreatus* (Rose 1973).

Although lungworms of ruminants dominate the literature on this metastrongyloid family, some research has been done on species of the genus *Protostrongylus* that infect hares and rabbits of North America, Europe and Asia. Boev (1975) listed seven species of leporid lungworms in a review of the genus, although taxonomy of this group is far from certain and there is no universal agreement on the number of valid species. The European and Asian forms have been studied best, and life cycles have been determined for most species of these lungworms. All follow the common protostrongylid trait of using a terrestrial gastropod intermediate host (Boev 1975). The natural intermediate hosts of some of these

species are known (Rizhikov 1956a; Rodonaya 1971), but temporal aspects of infection in snail hosts have not been studied. Preliminary investigation of some transmission aspects of at least one species of lungworm in a population of hares has been reported (Maklakhova 1979).

Little attention has been given the two known species of *Protostrongylus* in leporids of North America: *Protostrongylus sylvilagi* Scott, 1943 in Nuttall's cottontail (*Sylvilagus nuttalli*) and the white-tailed jackrabbit (*Lepus townsendii*) (Bergstrom and Honess 1982), and *Protostrongylus boughtoni* in the snowshoe hare (*Lepus americanus*). *P. boughtoni* was first described from Manitoba as *Synthetocaulus leporis* by Boughton (1932) and was amended to the current form by Goble and Dougherty (1943). Although it has been listed frequently in surveys of the definitive host, aside from pilot investigations by Pillmore (1957, 1959, 1961), the life cycle of this parasite had not been elucidated and details of the transmission of *P. boughtoni* in natural populations of hosts were unknown.

Lungworm-induced pathology has been postulated periodically as an influential factor in the cyclical decline of snowshoe hare populations (Boughton 1932; Erickson 1944; Bookhout 1971; Carey and Keith 1979). Similarly, heavy infections of *P. stilesi* and/or *P. rushi* are thought to predispose bighorn sheep to infection with pneumonia-causing organisms, significantly influencing mortality rates in free-ranging herds (see review of

Forrester 1971). Investigation of the transmission of *P. boughtoni* in a natural population of snowshoe hares may provide information of value to studies of other protostrongylid-host systems, as well as reveal adaptations which allow a suprapopulation (Esch *et al.* 1975) of parasites to survive in a system with an unstable definitive host population.

The present study was initiated to provide details on the natural transmission of *P. boughtoni*. A two-stage investigation was developed, integrating both a laboratory and field approach. Primary objectives of the study were to: 1) complete the life cycle of *P. boughtoni* experimentally, determining details of intra-host stages; 2) investigate the temporal dynamics of *P. boughtoni* infection in natural populations of intermediate and definitive hosts; and 3) describe the natural transmission of *P. boughtoni* using simple models adaptable to research on closely-related protostrongylids.

CHAPTER I. THE LIFE CYCLE OF PROTOSTRONGYLUS BOUGHTONI IN LABORATORY-REARED HOSTS

Introduction

A necessary first requirement for investigating the transmission of any parasite under natural conditions is an understanding of the details of that parasite's life cycle. Information on development in the intermediate and definitive hosts and output of immature stages from the definitive host must influence the design and timing of field investigations. Regardless of the objective, the elucidation of the life cycle of a parasite is best described by Olsen (1974) as, "a distinct challenge and a gratifying achievement".

Life cycles have been described for four of the five species of *Protostrongylus* infecting European leporids (Boev 1975), and one of the two species infecting North American leporids, *Protostrongylus sylvilagi* of *Sylvilagus nuttalli*, (Pillmore 1957,1959,1961). These species follow a typical protostrongylid life cycle pattern; that is, first-stage larvae expelled from the host in feces enter a land snail, generally within the families Valloniidae, Pupillidae, Helicellidae, Helicidae and Enidae (Pillmore 1956). After a period of development in the snail, the larvae reach the infective third-stage. Snails with infective larvae are apparently ingested by the leporids and patent infections are achieved after a further development period. The route of migration to the lung from the gastro-intestinal tract is

poorly known.

Not all metastrongyloid life cycles are indirect as described above. For example, *Oslerus osleri* and *Filaroides hirthi* of canids, and *Andersonstrongylus captivensis* of skunks may be transmitted directly by ingestion of first-stage larvae (Dorrington 1968; Georgi *et al.* 1976; Webster 1980, respectively). Prenatal infection has been demonstrated for *Protostrongylus stilesi* and/or *rushi* in bighorn sheep of North America (Hibler *et al.* 1972,1974; Gates and Samuel 1977; Kistner and Wyse 1979; Spraker 1979), and *Protostrongylus hobmaleri, Protostrongylus rallietí, Protostrongylus skrjabini* and *Cystocaulus ocreatus* in domestic sheep of Russia (Azimov and Kulmamatov 1978).

In the only reports concerning the life cycle of *Protostrongylus boughtoni*, the North American hare lungworm, Pillmore (1957,1959,1961) identified *Pupilla blandi* and *Vallonia pulchella* as intermediate hosts. Third-stage larvae were characterized by a "dark sheath" and four New Zealand white rabbits (*Oryctolagus cuniculus*) exposed *per os* to these did not become patent. No other details were provided.

The general objective of this chapter of the study was to provide details on the life cycle of *P. boughtoni*. Specific objectives were to: 1) describe all larval stages; 2) determine the mode of entry of first-stage larvae into the snail; 3) document developmental patterns of the larvae in the intermediate host; 4) investigate the possible emergence of third-stage larvae from the living snail; 5)

determine the prepatent and patent periods and pattern of larval output in the definitive host; and 6) investigate the possibilities of direct and prenatal transmission.

Materials and Methods

Development in the Intermediate Host

Method of Exposing and Caring for Snails

The valloniid snail, *Vallonia pulchella* Mueller, a species introduced to western Canada, was collected from a garden on the University of Alberta campus. A reproducing colony was maintained in a covered glass terrarium partly-filled with garden soil and Manitoba maple leaves (*Acer negrundo*). Snails were provided with lettuce and blackboard chalk, misted with water weekly and kept at room temperature (23°C). Another valloniid snail, *Vallonia gracilicosta* Reinhardt, was collected from a woodlot near Ellerslie, Alberta and maintained as above with the substitution of a poplar leaf/spruce needle (*Populus tremuloides/Picea glauca*) substrate.

To expose snails to *P. boughtoni*, first-stage larvae (L1's) were recovered from the lungs of snowshoe hares captured near Slave Lake, Alberta as outlined in Chapter II. Ten-thousand L1's, in a minimum amount of distilled water, were spread evenly over a filter paper (Whatman #1) disc in the bottom of a 60mm glass stendor dish. Fifty snails were placed on the filter paper and the dish was covered. Snails were exposed for 2 1/2 hours and during this period any individuals that crawled up the sides of the dish were

placed back on the filter paper.

Exposed snails were kept in 12.4cm, covered finger bowls containing sterilized soil, maple leaves, chalk and lettuce. They were misted frequently, and maintained at 18±1°C.

Mode of Entry

The mode of entry of L1's into *V. pulchella* was investigated as follows. Fifty snails were exposed to L1's as outlined above, then five were immediately fixed in hot Bouin's. They were prepared for histological examination following standard procedures, sectioned at 7um and stained in Harris' hematoxylin and eosin (H&E). All sections of feet and viscera were examined for first-stage larvae. Nineteen snails were killed and examined for larvae 40 days later.

Larval Development

To investigate the growth of *P. boughtoni* in *V. pulchella*, two snails were collected at 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, and 34 days post-exposure (d.p.e.). Each snail was crushed, the foot removed, and larvae teased from the tissue into 0.85% saline with forceps and a probe. Larvae were killed and fixed in hot glycerin-alcohol (95 parts 70% EtOH, 5 parts glycerin). The fixative was evaporated to pure glycerin and the cleared larvae were examined as whole mounts. Lengths and widths of ten larvae from each day were measured in micrometers (um) using a drawing tube and measuring wheel.

Larval Emergence From Snails

To determine the rate at which third-stage larvae (L3's) left the intermediate host, larvae in the foot of 12 individual *V. pulchella* were counted (34 d.p.e.) at 40X using transmitted light. Snails were maintained at room temperature in individual 32ml glass vials containing a small piece of lettuce. At weekly intervals for 10 weeks, the L3's in the foot of each snail were counted and the surfaces of the vial and lettuce were examined at 16X for larvae.

Description of Larvae

First-, second-, third- and fourth-stage larvae were prepared as previously outlined and 20 of each were measured in more detail for taxonomic description. First-stage larvae were recovered from the feces of an experimentally infected hare by zinc-sulfate flotation (see below). Second- and third-stage larvae were recovered from infected snails as outlined above, and fourth-stage larvae were recovered from the lungs of naturally-infected snowshoe hares (Chapter II). Specimens of all larval stages of *P. boughtoni* were deposited in the National Museum of Canada Invertebrate Collection (Parasitology) (NMCIC(P)) and also given a University of Alberta Parasitology Collection (UAPC) catalogue number. In addition, 20 L3's of *Orthostrongylus macrotis* from mule deer and *Protostrongylus* spp. (*stilesi* and/or *rushi*) from bighorn sheep were obtained from *V. pulchella* exposed previously to L1's recovered from infected hosts and measured for comparison with L3's of *P. boughtoni*.

Drawings were made with the aid of a drawing tube. All measurements are given in micrometers (um); the range is followed by the mean and standard deviation.

Suitability of V. pulchella as an Intermediate Host

Three criteria were used to determine the suitability of *V. pulchella* as an intermediate host for *P. boughtoni*. First, a comparison of the tissue reaction to infective larvae of *P. boughtoni* was made between laboratory-infected *V. pulchella* and naturally-infected *Vertigo gouldi* Binney, a locally-occurring intermediate host. Two *V. pulchella*, exposed 39 days previously to *P. boughtoni*, fixed in hot Bouins', sectioned at 7um and stained in H&E were compared to two naturally-infected *V. gouldi* that were prepared similarly.

The second method consisted of a statistical comparison of the lengths of 20 L3's from *V. pulchella* with 19 L3's from naturally-infected *V. gouldi*. Larvae from both snails were recovered and examined as outlined above.

The third method was a comparison of the pre-infective period of *P. boughtoni* larvae in *V. pulchella* and *V. gracilicosta*, another locally-occurring, natural intermediate host. Fifty *V. gracilicosta* were exposed to *P. boughtoni* as outlined above and examined daily for presence of L3's.

Data Analysis

Statistical analyses were accomplished using the programs in MIDAS (Fox and Guire 1976). Non-parametric

statistics were used in cases of heterogeneity of variance between sample groups that could not be corrected by transformation of variables. Otherwise, parametric statistics were applied.

Development in the Definitive Host

Care, Exposure and Fecal Examination of Definitive Hosts

In May, June and July of 1981, pregnant snowshoe hares were live-trapped at the Slave Lake study site (see Chapter II) and transported to the University of Alberta Biomedical Animal Centre at Ellerslie, Alberta where they were held in individual outdoor wire-bottomed pens constructed after Bursey (1977). Hares were fed baby rabbit pellets[1] *ad lib.*, supplemented daily with 1/2 apple per individual and weekly with 1 teaspoon of powdered vitamin supplement[2], and given constant access to water, and to soil which they often ate.

Litters remained with their mothers for 25-30 days post-parturition, after which each juvenile hare was removed to an individual cage. Cages had wire-bottoms and sides, and were 0.66m x 2.29m x 0.94m high. Black polyethylene sheeting covered the back half of the cages. Each cage was provided with a removable nesting tray filled with straw. Once in individual cages, young were fed as outlined above. Feces from each animal were examined for larvae of *Protostrongylus* by zinc-sulphate flotation (see below).

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[1]Master Feeds, Maple Leaf Mills Ltd., Edmonton, Alta.
[2]Pervinal. St. Aubrey Division of 8 in 1 Pet Products Inc., New York

Rabbits (*Oryctolagus cuniculus*) were maintained in the University of Alberta Bioscience Animal Services facilities. They were kept in individual cages in rooms with a 12 hour photoperiod and fed baby rabbit pellets supplemented with carrots. Water was available *ad lib.*

Third-stage larvae for experimental infections were obtained from *V. pulchella* previously exposed to L1's recovered from wild-caught hares (see above). Snails were crushed and placed in artificial digest medium (0.6g pepsin, 0.7ml HCl, 100ml distilled water) in a 15x125mm test tube. The test tube was set in a water bath at 37°C and agitated frequently. After three hours of incubation, the test tube was centrifuged at 600 r.p.m. for five minutes, the supernatant was drawn off, and the plug of debris and L3's was washed repeatedly with saline by recentrifugation.

Animals were exposed *per os* to infective larvae in saline. Larvae were drawn into a 20cm length of polyethylene infusion tubing in a minimum of saline using a 1ml syringe. The tubing was put into the mouth of a restrained animal and pushed as far down the esophagus as possible. Larvae were slowly delivered from the tubing, allowing sufficient time for the animal to swallow. The procedure was repeated twice more with saline rinses to ensure that all larvae were eliminated from the tubing.

Feces of exposed animals were examined for larvae by zinc-sulfate flotation (Faust *et al.* 1938). Approximately one gram of pellets was weighed and soaked in 7ml of water

in a 15x125ml test tube for two hours. The pellets were poured into a 100ml beaker, crushed with a tongue depressor, and poured through a 7cm diameter tea strainer (approx. 0.7mm² mesh) into another beaker. This fluid was poured back into the test tube, centrifuged at 600 r.p.m. for five minutes and the supernatant removed. The sediment was resuspended in zinc-sulfate solution (sp. g.=1.18) which was added to the tube in droplets to form a positive meniscus. An 18mm square coverslip was gently placed on the tube which was then centrifuged at 600 r.p.m. for five minutes.

The coverslip with a drop of surface fluid was placed on a glass slide and examined at 40x. All larvae on the slide were counted, and a value for larvae per gram (LPG) of feces calculated.

Experimental Infection of Hares

Seven juvenile hares from two litters were anaesthetized with an intramuscular injection of a mixture of Atravet[3] and Ketaset[4] (1:10, 0.08ml/kg). Each was exposed to 50 L3's of *P. boughtoni*. One hare from each of the two litters was examined before the experiment to determine whether transplacental or direct transmission had occurred. The histories of animals infected are given in Appendix I.

Feces of exposed animals were examined daily beginning 21 d.p.e. until 21 days post-patency after which examinations were made twice-weekly until the end of

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[3]Ayerst, Montreal, Que.
[4]rogar/STB, London, Ont.

patency. Hares were killed within two days of the end of patency by cervical fracture and the lungs were examined for adult nematodes (see Chap. II). First-stage larvae were recovered from lung tissue as outlined in Chapter II. Lung tissue was fixed in 10% neutral buffered formalin, sectioned at 7um and stained in H&E. In addition, lung tissue from wild-caught hares was prepared similarly to identify development sites of juvenile (early L5) *P. boughtoni*.

Experimental Infection of Rabbits

Two domestic *O. cuniculus*, Dutch Belted strain, were exposed to 50 L3's of *P. boughtoni* each. Two *O. cuniculus* were exposed to 5,000 L1's each of *P. boughtoni* from the lungs of a wild-caught hare (Chapter II) in the same manner. Feces were examined daily beginning 21 d.p.e. until the end of patency for the L3 exposures, and every second day from 21 d.p.e. for the L1 exposures.

One rabbit was exposed to 23 L3's of *P. boughtoni* which had emerged from infected *V. pulchella*. Feces were examined daily until patency, after which the rabbit was terminated.

All rabbits were approximately 3 months old when exposed. Rabbits were killed by Euthanol[5] injection and lungs were examined for adult nematodes. Lung tissue was prepared for histological examination as outlined above.

Transplacental Transmission

Dying, captive-born hares, stillborn hares, and hares dying *in utero* were examined for the presence of *P.*

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[5]MTC Pharmaceuticals, Hamilton, Ont.

boughtoni. Prenatal hares were aged using characters described by Bookhout (1964). Data from hares captured in 1980 from the Slave Lake study area are included, and details of all hares are given in Appendix II.

The liver and lungs of stillborn hares and the liver, lungs, and placental discoid of hares dying *in utero* were macerated in artificial digest solution which was incubated for 12 hours. The resultant sediment was examined at 16X for larvae. The lungs of older captive-born hares were teased apart and examined for nematodes at 6.4X.

Results

Development in the Intermediate Host

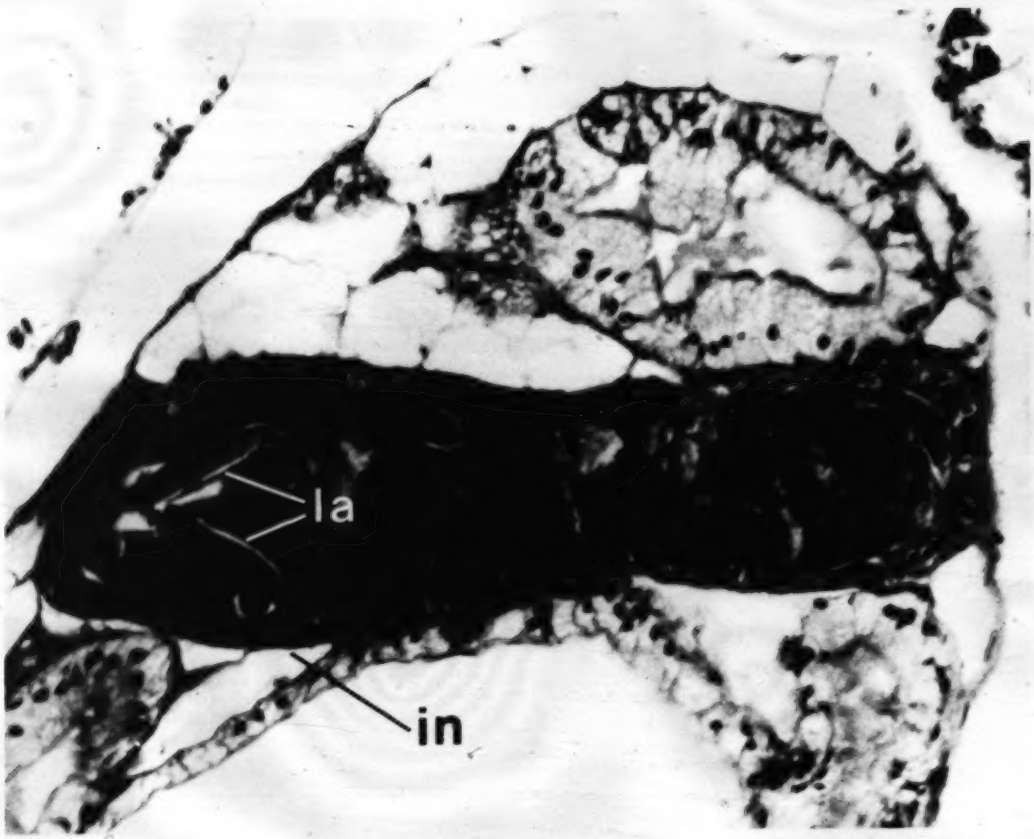
Mode of Entry

First-stage larvae were found in the esophagus, stomach and pro- to mid-intestine (microanatomy after Pan 1958) of each *V. pulchella* after the 2 1/2 hour exposure period (Fig. 1). No L1's were found in the feet. None of the larvae observed penetrated the gut epithelium, and none were outside the lumen.

Single larvae were observed in an average of six consecutive sections. L1's were thus counted in every fifth section, and the totals were summed for each snail (see Platt 1978). There were 17, 27, 42, 44 and 77 L1's in the alimentary tracts of the five snails. Nineteen other snails exposed concurrently with these five contained a mean of 16.7 L3's (range=11-27) in the foot after 40 days.

Larval Development

Figure 1. First-stage larvae of Protostrongylus boughtoni in the intestine of Vallonia pulchella after 2½ hours of exposure. 90X. (in - intestine, la - larvae).


la
in

Growth of larval *P. boughtoni* was difficult to detect prior to the first molt in snail tissue (Fig. 2). The first molt began on day 14 (7L1:3L2) and was nearly complete by day 18 (1L1:9L2). The period between the first and second molt was characterized by rapid growth, greater variance in length at each period, and an increasingly granular appearance of the intestine. The rate of growth declined somewhat after day 22; the second molt began on day 28 and was complete by day 30. Maximum length was essentially achieved by day 30 and average width decreased slightly after the second molt.

Larval Emergence From Snails

A total of 30 L3's of *P. boughtoni* emerged from 6/12 infected *V. pulchella* (Table I). Of these, 16 were found on the plastic vial cap, 13 were found on the lettuce and 1 was found on the glass wall of the vial. Isolated larvae were first found after the second week of the experiment (48 d.p.e.), and they continued to emerge sporadically throughout the 10 week period.

Seven snails died five to seven weeks into the experiment. All L3's remained in the foot of the decaying snails. In four of five survivors there was a reduction in intensity of infection of up to 87.5% after 10 weeks. The rate at which L3's emerged varied from 0 to 1.4/wk among the snails. No snails were freed of infection over the period of the experiment.

Figure 2. Larval development of *Protostrongylus boughtoni* in experimentally-infected *Vallonia pulchella* at 18°C. Bars above and below points represent 1 standard deviation, and arrows indicate the beginning of the first and second larval molt, respectively.

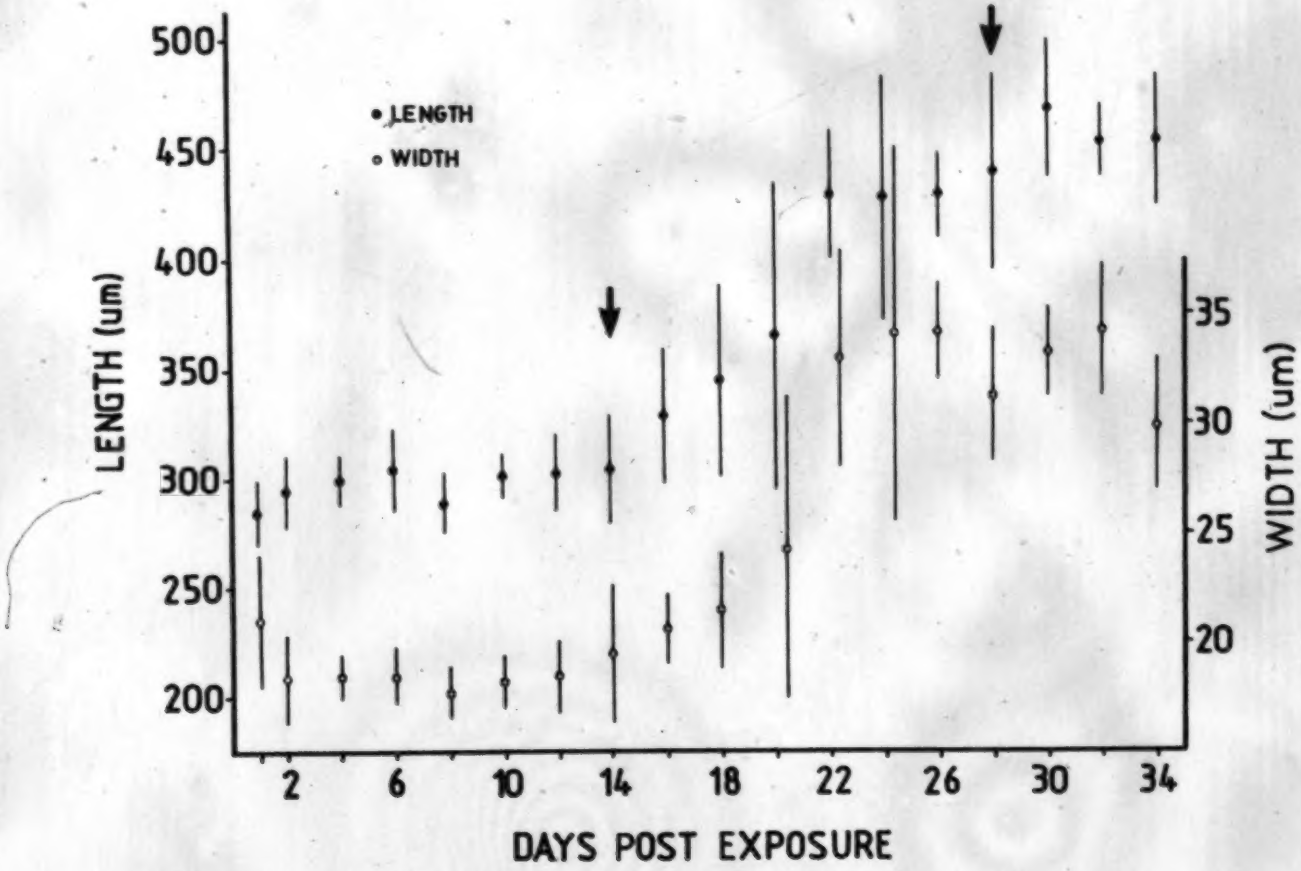

LENGTH
WIDTH
500
450
400
350
300
250
200
LENGTH (um)
35
30
25
20
WIDTH (um)
2
6
10
14
18
22
26
30
34
DAYS POST EXPOSURE

TABLE I. NUMBER OF THIRD-STAGE LARVAE OF *PROTOSTRONGYLUS BOUGHTONI* EMERGING FROM INFECTED *VALLONIA PULCHELLA* AT WEEKLY INTERVALS

SNAIL NUMBER	L3's IN FOOT 34 DAYS POST-EXPOSURE	NUMBER OF L3's EMERGED DURING WEEK: 1	2	3	4	5	6	7	8	9	10	TOTAL L3's EMERGED	% L3's EMERGED	L3's/wk EMERGED
1	12	-	-	2	3	-	-	-	-	2	-	7	58.3	0.7
2	14	-	-	1	-	dead						1	7.1	0.3
3	15	-	-	-	-	-	-	-	-	-	-	0	0	0
4	13	-	-	-	-	dead						0	0	0
5	16	-	-	-	-	1	12	-	-	1	-	14	87.5	1.4
6	5	-	1	-	-	-	-	2	-	-	-	3	60.0	0.3
7	8	-	-	-	-	-	1	dead				1	12.5	0.1
8	3	-	-	-	-	-	dead					0	0	0
9	6	-	1	-	2	-	1	-	-	-	-	4	66.7	0.4
10	12	-	-	-	-	-	dead					0	0	0
11	17	-	-	-	-	dead						0	0	0
12	17	-	-	-	-	-	-	dead				0	0	0
TOTALS	138	-	2	3	5	1	14	2	-	3	-	30	21.7	

In two snails, L3's migrated from the foot to the viscera within the shell after 4 weeks. In one of these, an L3 migrated from the viscera and to the dorsal foot tissue after 10 weeks. L3's in the other 10 snails were found only in the foot.

Description of Larvae

Protostrongylus boughtoni Goble and Dougherty, 1943

Synonyms: *Synthetocaulus leporis* Boughton, 1932; *Synthetocaulus cuniculi* Green and Shillinger, 1935; *Protostrongylus leporis* MacLulich, 1937.

Hosts: *Lepus americanus* Erxleben, *Sylvilagus floridanus mallurus* (Thomas).

Location: Bronchi and bronchioles.

Localities: Canada (New Brunswick, Quebec, Ontario, Manitoba, Alberta) and United States (Maine, New York, Michigan, Minnesota, Wisconsin, Colorado, Alaska).

First-Stage Larva (Fig. 3; Table II)

Body tapering at both ends, 253-307 (282±14) long and 11-16 (14.6±1.0) wide at the level of the esophagus-intestine junction. Nerve ring 54-70 (64±5) from anterior end. Excretory pore 62-79 (72±4) from anterior end with duct leading to paired subventral excretory glands containing prominent nuclei. Esophagus 107-134 (122±8) long with distinct procorpus, metacorpus, isthmus and bulbar

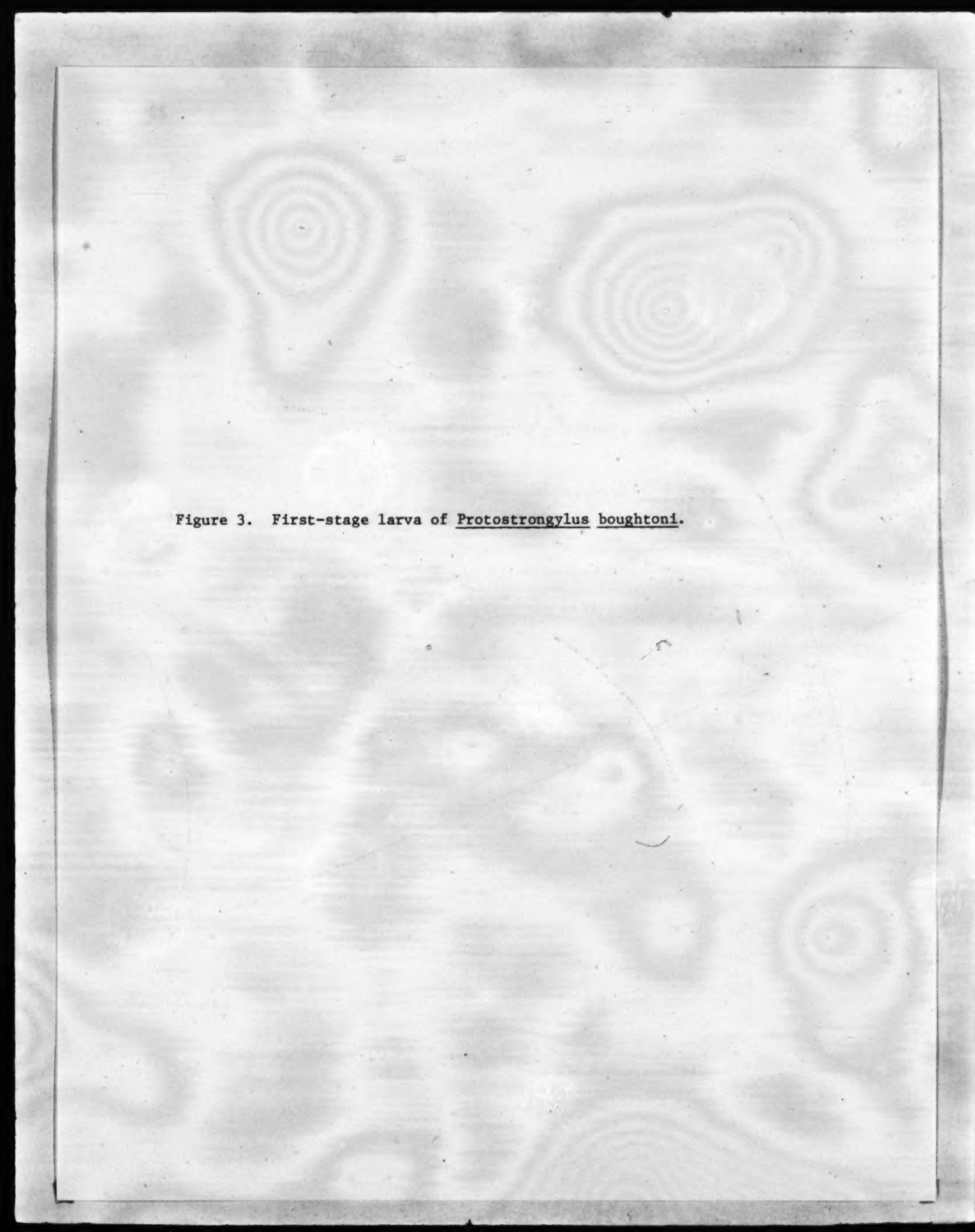


Figure 3. First-stage larva of Protostrongylus boughtoni.

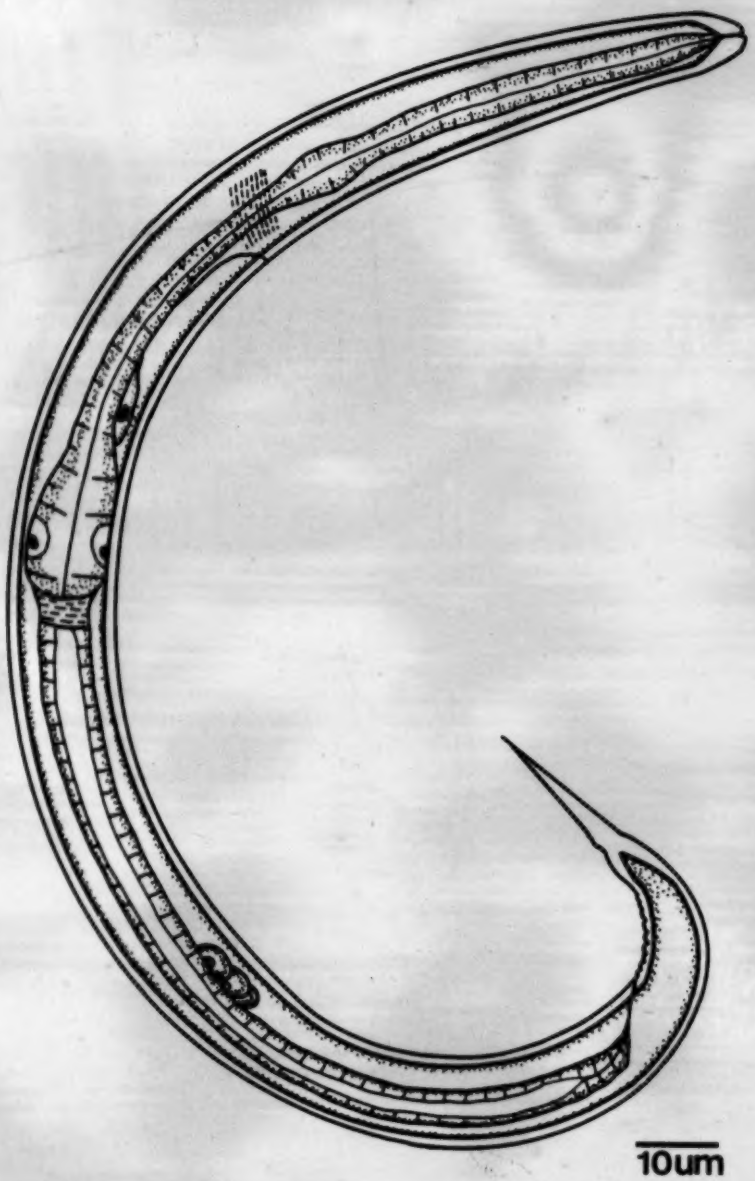

10um

TABLE II. COMPARATIVE MEASUREMENTS* OF FIRST-STAGE *PROTOSTRONGYLUS* LARVAE FROM LEPORIDS AND *PROTOSTRONGYLUS*/*ORTHOSTRONGYLUS* SPECIES FROM NATIVE NORTH AMERICAN RUMINANTS

	P. boughtoni			*P. cuniculorum*	*P. pulmonalis*	*P. tauricus*
	Goble and Dougherty 1944	Pillmore 1956	This Study	Joyeux and Gaud 1946	Gvozdev 1948	Shults and Kadenatsii 1949
n =	?	5	20	?	?	?
Fixative	?	gly-alc	gly-alc	?	?	?
Length	320-360	282-302	253-307	315-370	243-280	396-500
Width	14-16	18-18	11-16	23-28	16-23	24-27
Nerve Ring	-	-	54-70	-	-	78
Excretory Pore	-	74-80	62-79	85-96	-	119-127
Esophagus	-	123-128	107-134	-	90	184-197
Genital Primordium	-	173-188	155-192	-	-	284
Anus	-	242-257	212-260	278-327	262	351-438

*in micrometers

TABLE II. CONTINUED

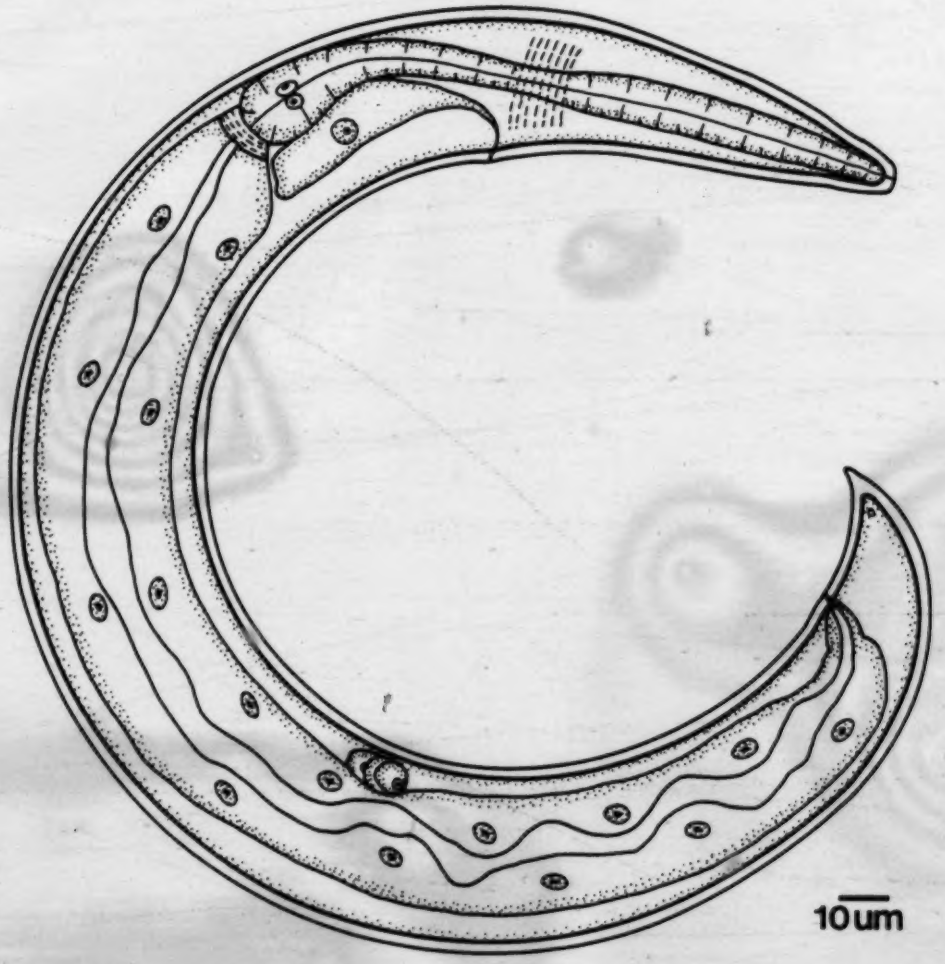
	P. kamenskyi + *P. pulmonalis* (mixed)	*P. rushi*	*P. stilesi*		*O. macrotis*
	Rizhikov *et al.* 1956a	Pillmore 1956	Pillmore 1956	Monson and Post 1972	Pillmore 1956
n =	?	5	25	50	5
Fixative	?	living	living	gly-alc	gly-alc
Length	340-350	336-371	286-371	198-368	242-256
Width	16-19	19-20	16-19	10-25	14-16
Nerve Ring	-	-	-	-	-
Excretory Pore	-	96-104	72-104	-	59-67
Esophagus	130-140	144-152	110-155	-	107-128
Genital Primordium	-	208-240	166-225	-	151-180
Anus	289-296	295-320	241-306	-	215-224

regions. Nuclei of dorsal and subventral esophageal glands prominent. Esophago-intestinal valve well-developed. Intestine thin-walled, agranular, terminating ventrally at anus 212-260 (237±13) from anterior end. Genital primordium trilobed with prominent nuclei, 8.1-11.5 (9.7±0.8) long by 2.7-6.1 (4.4±0.8) wide and 155-192 (173±9) from the anterior end. Cuticle with minute transverse striations over entire midsection (Fig. 3 does not show). Paired lateral alae extend from near the level of the nerve ring to the base of the tail. Tail terminating in sharp point. Dead larva generally assumes "C" shape. Specimens deposited under catalogue numbers NMCIC(P)1983-0006 and UAPC10675.

Second-Stage Larva (Fig. 4)(26 d.p.e)

Body tapering at both ends, 407-485 (439±21) long and 30-42 (35±3) wide at the level of the esophagus-intestine junction. Nerve ring 57-83 (72±7) from anterior end. Excretory pore 78-97 (89±6) from anterior end with duct leading to paired subventral excretory glands relatively enlarged from first-stage, nuclei still prominent. Esophagus 113-139 (126±8) long, metacorpus indistinct compared with first-stage. Nuclei of dorsal and subventral esophageal glands prominent. Esophago-intestinal valve well-developed. Intestine thick-walled with 8 nuclei visible in either side of wall, terminating ventrally at anus 381-461 (414±21) from anterior end. Intestine containing refractile granules (not shown in Fig. 4). Genital primordium exhibiting little

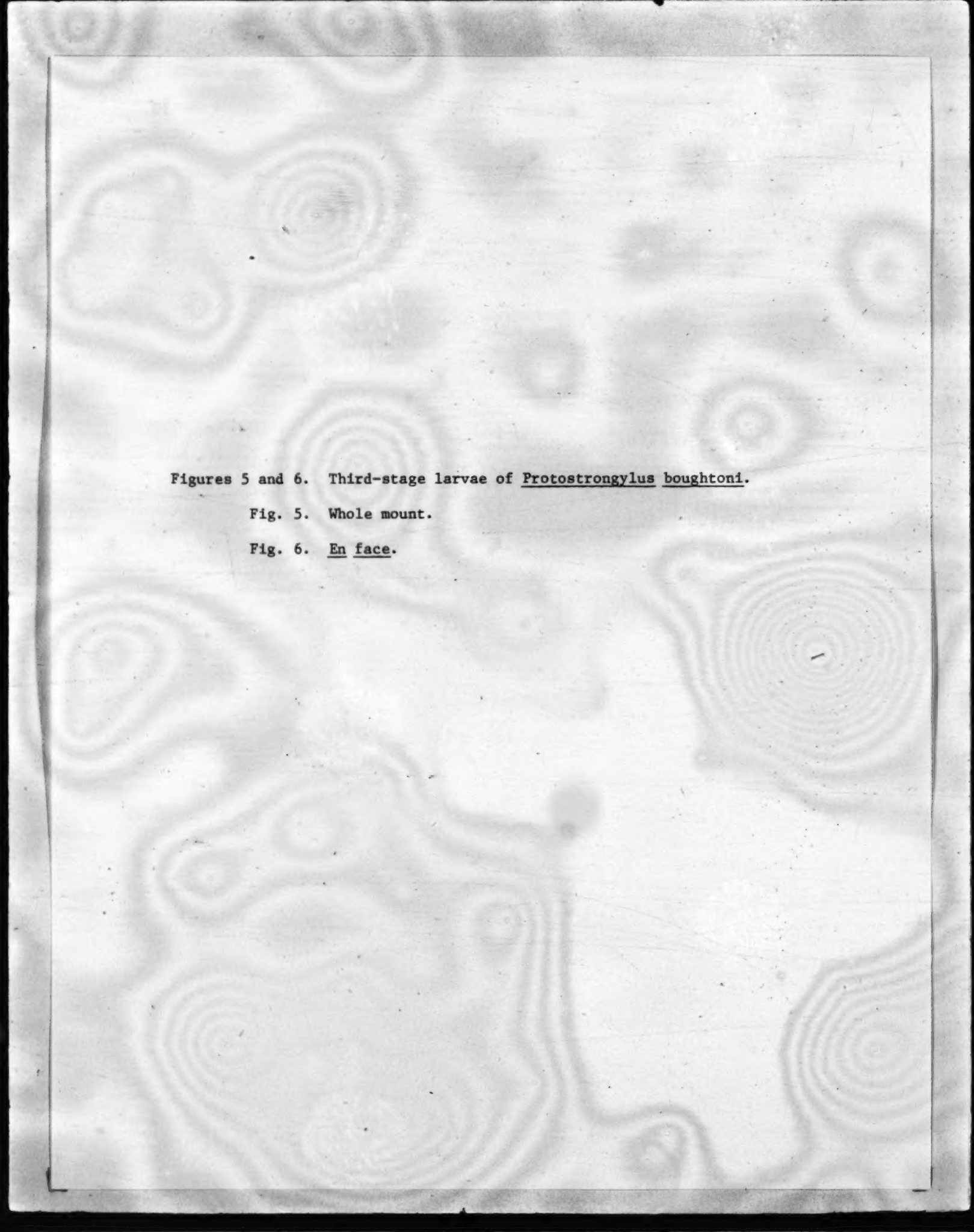
Figure 4. Second-stage larva of Protostrongylus boughtoni.


10um

development from first-stage, 9.9-20.0 (14.1±3.0) long by 5.4-11.7 (8.3±1.4) wide and 263-314 (287±15) from the anterior end. Transverse cuticular striations of midsection much reduced from first-stage. Lateral alae absent. Phasmids present at tail base. Tail bluntly-pointed, simple. Entire larva ensheathed by transparent cast-off first-stage cuticle. Living larva generally assumes "O" shape in snail tissue. Specimens deposited under catalogue numbers NMCIC(P)1983-0007 and UAPC10676.

Third-Stage Larva (Figs. 5,6,7,8; Table III)(30 d.p.e.)

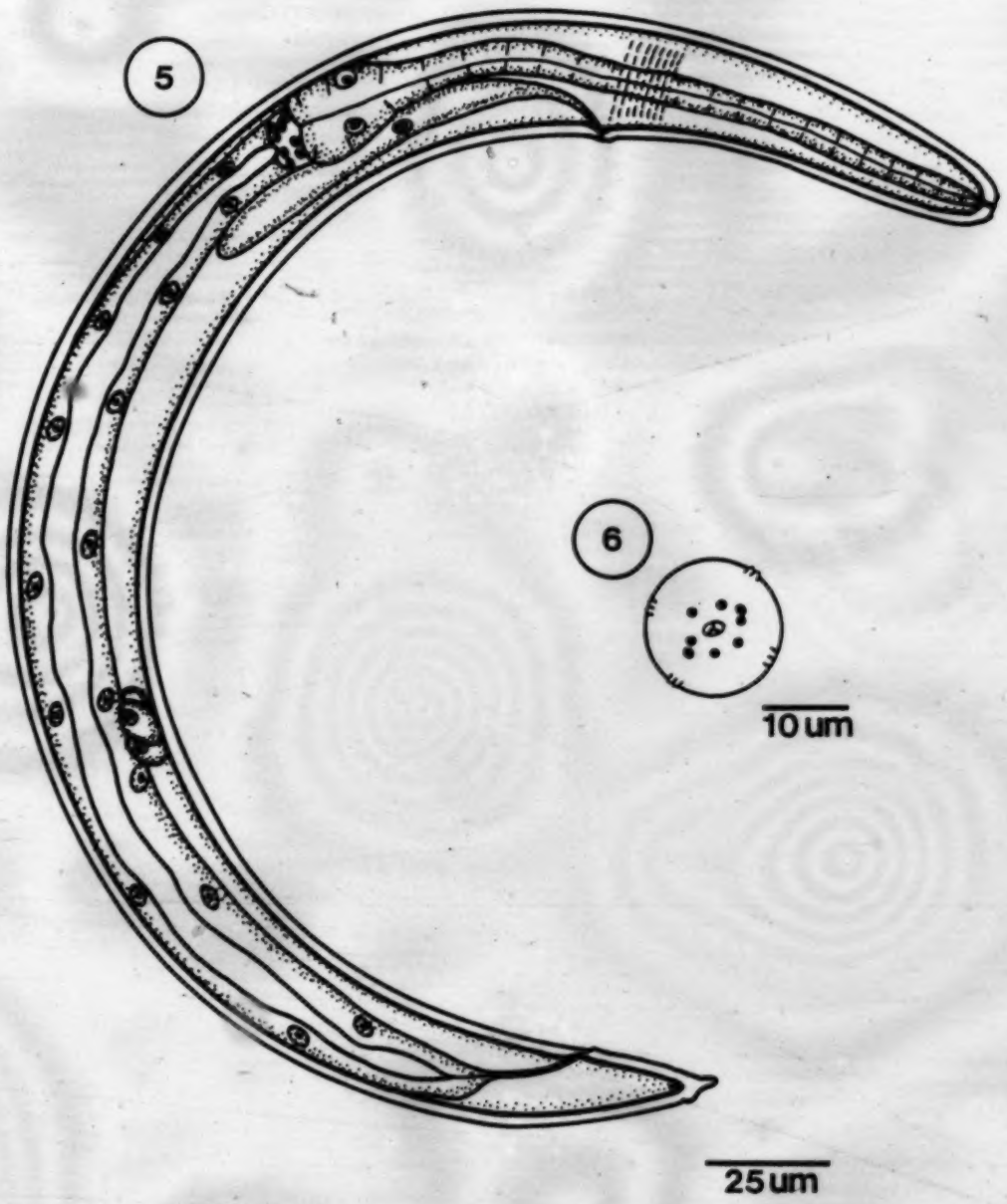
Body tapering at both ends, 381-500 (430±30) long and 20-30 (26±2) wide at the level of the esophagus-intestine junction. Nerve ring 58-93 (74±8)from anterior end. Excretory pore 80-107 (90±7) from anterior end with duct leading to paired subventral excretory glands extending posterior to esophago-intestinal valve and containing prominent nuclei. Esophagus 129-192 (152±14) long with indistinct metacorpus, nuclei of dorsal and subventral esophageal glands prominent. Numerous nuclei of esophago-intestinal valve visible. Intestine relatively thin-walled compared with second-stage, agranular, terminating ventrally at anus 358-472 (405±28) from anterior end. Eight nuclei visible in either side of intestinal wall. Genital primordium exhibiting little development from second stage, 14.2-27.0 (18.3±2.8) long by 6.1-10.1 (7.9±1.4) wide and 239-334 (278±23) from the anterior end. Anterior end



Figures 5 and 6. Third-stage larvae of *Protostrongylus boughtoni*.

Fig. 5. Whole mount.

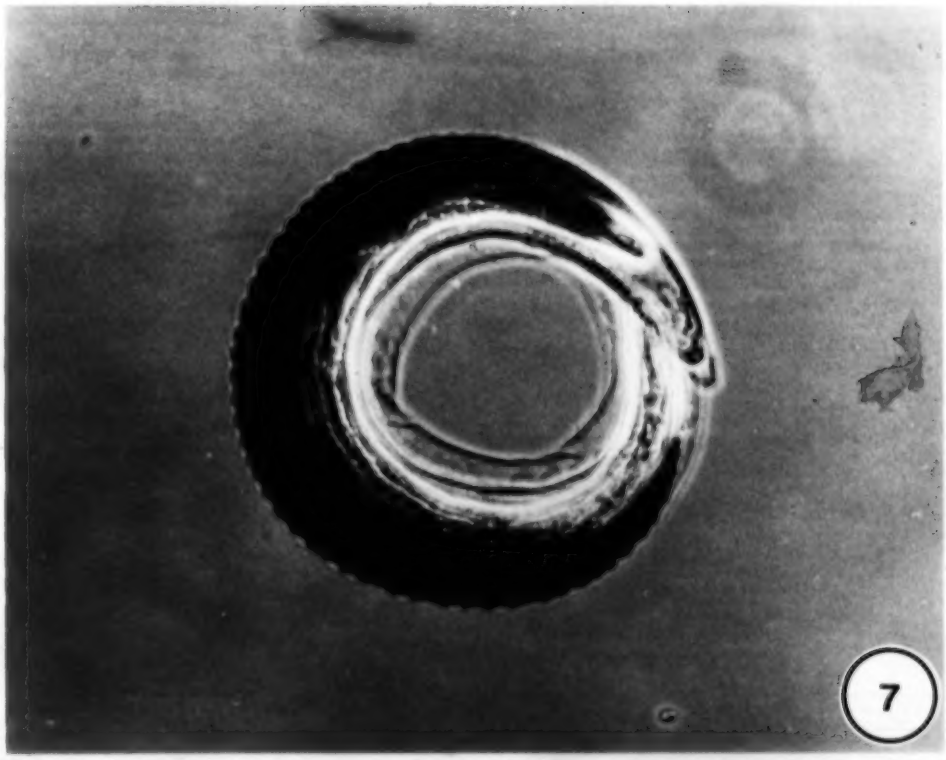
Fig. 6. *En face*.


5
6
10 um
25 um

Figures 7 and 8. Third-stage larvae of Protostrongylus boughtoni.

Fig. 7. Whole mount. 300X.

Fig. 8. Larvae in foot of Vallonia pulchella. 20X.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8

TABLE III. COMPARATIVE MEASUREMENTS* OF THIRD-STAGE *PROTOSTRONGYLUS* LARVAE FROM LEPORIDS AND *PROTOSTRONGYLUS*/*ORTHOSTRONGYLUS* SPECIES FROM NATIVE NORTH AMERICAN RUMINANTS

	P. boughtoni This Study	*P. cuniculorum* Joyeux and Gaud 1946	*P. kamenskyi* + *P. pulmonalis* (mixed) Rizhikov *et al.* 1956a	*P. tauricus* Kadenatsii 1969
n =	20	?	?	?
Fixative	gly-alc	?	?	?
Length	381-500	550-750	500-640	540-670
Width	20-30	35-55	51-58	44
Nerve Ring	58-93	-	-	-
Excretory Pore	80-107	115-135	-	-
Esophagus	129-192	230-265	-	220
Genital Primordium	239-334	420-465	-	-
Anus	358-472	-	-	496-626

*in micrometers

TABLE III. CONTINUED

	P. stilesi and/or *rushi* This Study	*O. macrotis* This Study
n =	20	20
Fixative	gly-alc	gly-alc
Length	527-673	488-611
Width	30-48	31-43
Nerve Ring	-	-
Excretory Pore	-	-
Esophagus	-	-
Genital Primordium	-	-
Anus	-	-

possesses six papillae of the internal circle with paired, opposite amphids immediately external, and four double, submedian papillae of the external circle. Transverse cuticular striation absent, narrow lateral alae present. Phasmids present at tail base. Tail bluntly-pointed with small, dorsal swelling. Entire larva ensheathed by two molted cuticles: transparent second-stage cuticle immediately external and the first-stage cuticle outer. Several days after the second molt, the first-stage cuticle acquires a rugose, dark brown appearance. Living larvae assume "O" or "C" shape in snail tissue and are motile. Specimens deposited under catalogue numbers NMCIC(P)1983-0008 and UAPC10677.

Fourth-Stage Larva (Fig. 9)

Body tapering at both ends, 453-614 (511±44) long and 28-41 (34±3) wide at the level of the esophagus-intestine junction. Nerve ring 68-111 (86±11) from anterior end. Excretory pore 92-125 (106±9) from anterior end with duct leading to paired subventral excretory glands extending posterior to esophago-intestinal valve and containing prominent nuclei. Esophagus 125-200 (158±18) long with swollen corpus compared to third-stage. Nuclei of dorsal and subventral esophageal glands prominent. Numerous nuclei of esophago-intestinal valve visible. Intestine thin-walled, containing dark brown ingested material, terminating ventrally at anus 426-578 (485±42) from anterior end. Ten

Figure 9. Fourth-stage larva of Protostrongylus boughtoni.

25um

nuclei visible in either side of intestinal wall. Genital primordium with extensive anterior and posterior development from third-stage, 253-430 (325±48) long by 8-16 (12±2.5) wide and 67-131 (95±23) from the anterior end. Transverse cuticular striation absent. Tail bluntly-pointed with small, dorsal swelling. Larvae generally assume "C" shape when dead. Specimens deposited under catalogue numbers NMCIC(P)1983-0009 and UAPC10678.

Suitability of V. pulchella as an Intermediate Host

Third-stage larvae in *V. gouldi* were surrounded by a thin cyst wall composed of one to several layers of flattened amoebocytes (Figs. 10,11). There were no amoebocytes within the cyst, only the larva surrounded by empty space.

The tissue response of *V. pulchella* to L3's was similar. A simple cyst wall surrounded the larvae and empty space (Figs. 12,13). There was no evidence of amoebocytic infiltration within the cyst or cellular proliferation outside the cyst boundary.

There was no significant difference (Mann-Whitney U=148; $p>0.05$) between the lengths of *P. boughtoni* L3's developing in *V. pulchella* (mean length=430um) compared with *P. boughtoni* developing in *V. gouldi* (mean length=445um)

Third-stage larvae with darkened cuticles were visible 30 d.p.e. in the feet of 5/10 *V. pulchella*. Darkened L3's were visible in the feet of 4/11 *V. gracilicosta* after the

Figures 10-13. Tissue response of snails to third-stage larvae of *Protostrongylus boughtoni* in the foot.

Fig. 10. L3 in *Vertigo gouldi*. 325X. (cw - cyst wall, la - larva, pg - pedal gland).

Fig. 11. L3 in *Vertigo gouldi*. 325X. (cw - cyst wall, la - larva).

Fig. 12. L3 in *Vallonia pulchella*. 325X. (cw - cyst wall, fe - foot epithelium, la - larva).

Fig. 13. L3 in *Vallonia pulchella*. 325X. (cw - cyst wall, fe - foot epithelium, la - larva).

pg
la
cw
10
11
fe
la
12
fe
la
cw
13

same period of time, indicating little difference between rates of development in the two snail species, one a natural intermediate host, the other introduced.

Development in the Definitive Host

Experimental Infection of Hares

Patent infections occurred in 6 of 7 juvenile hares given 50 L3's of *P. boughtoni* (Table IV). The animal that did not become patent, S.H. 28-4, was killed at 17 d.p.e. after severely injuring itself. Three male and two female *P. boughtoni* adults were recovered from the lungs. One small (<1cm) lesion was present on the surface of the intermediate lobe resulting from ova within the alveoli (Fig. 14). No first-stage larvae were found in the lungs.

Infections in the remaining hares became patent between 25 and 27 d.p.e. (Table IV). The patent period of infection varied between 41 and 104 days. Neither hare killed before the experiment was infected with *P. boughtoni*.

S.H. 28-3 was terminated at 19 days post-patency after an injury. Three male and three female *P. boughtoni* adults were recovered from the lungs, which also contained 127 LPG of tissue. First-stage larvae, as well as ova, were found in small (<1mm) lesions on the surfaces of the right diaphragmatic lobe (Fig. 15). Specimens of adult nematodes from experimental infections were deposited under catalogue numbers NMCIC(P)1983-0004 and UAPC10673.

The numbers of L1's recovered from feces of the 6 juvenile hares followed two major patterns through the

TABLE IV. EXPERIMENTAL INFECTIONS OF LEPUS AMERICANUS WITH PROTOSTRONGYLUS BOUGHTONI

HARE #	# L_3's RECEIVED	PREPATENT PERIOD	PATENT PERIOD	ADULT WORMS RECOVERED
S.H. 21-1	50	25d	81d	0
S.H. 21-2	50	27d	104d	0
S.H. 21-3	50	27d	62d	0
S.H. 21-4	50	27d	90d	1♂, 1♀
S.H. 28-1	50	27d	41d	0
S.H. 28-3*	50	26d	-	3♂, 3♀
S.H. 28-4**	50	-	-	3♂, 2♀

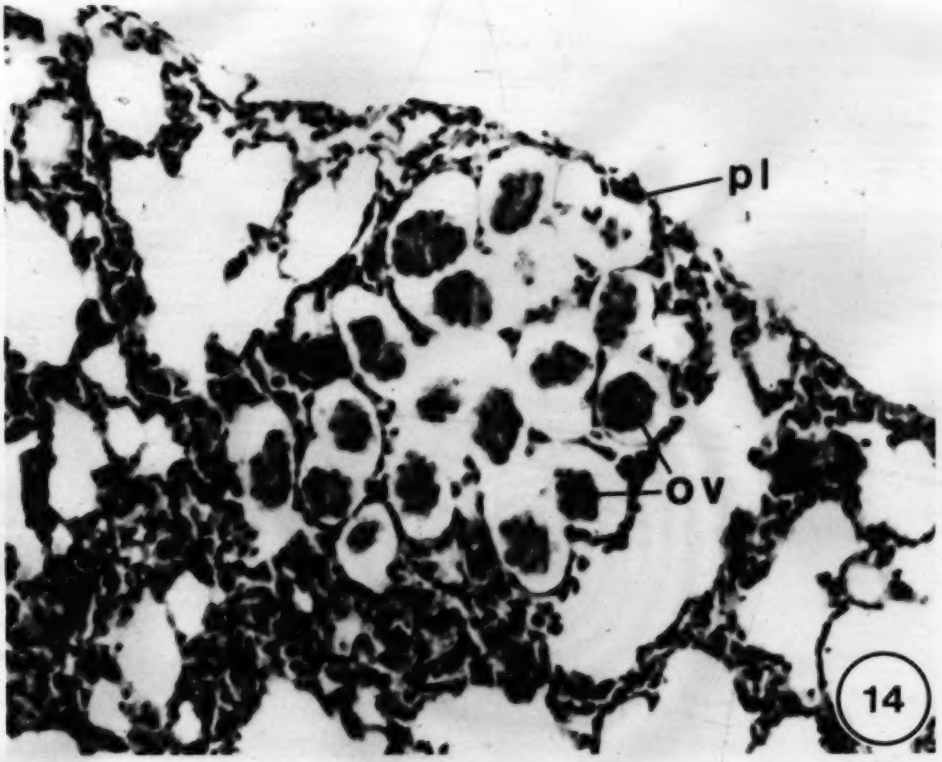
*Terminated on 19th day of patency.

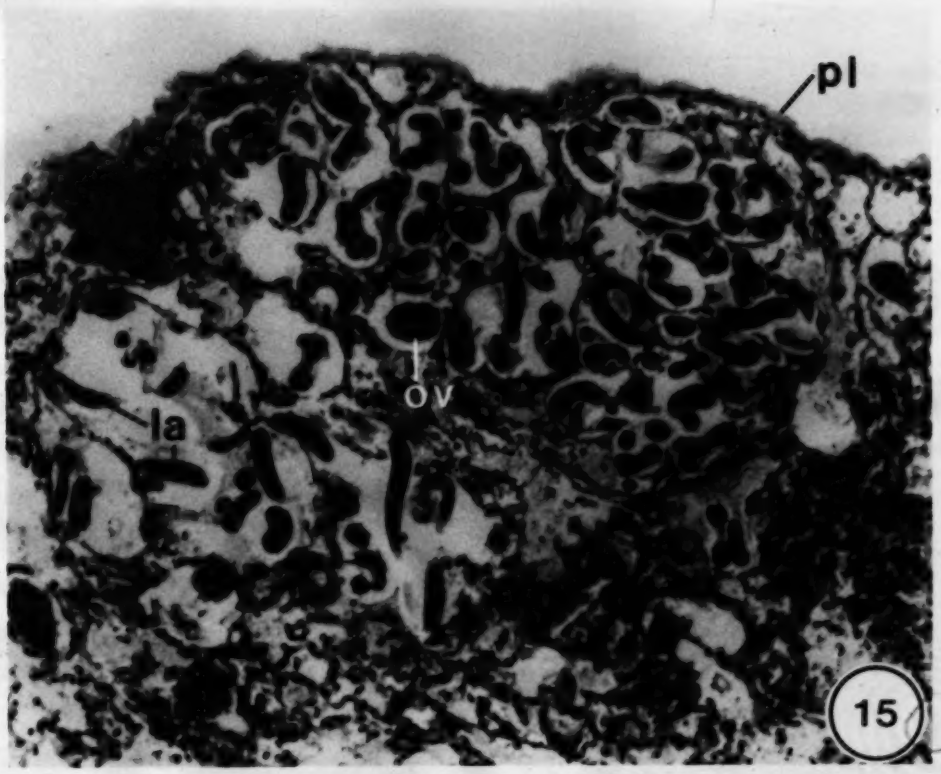
**Terminated at 17 days post-exposure.

Figures 14 and 15. Ova and larvae of *Protostrongylus boughtoni* in the lungs of experimentally-infected *Lepus americanus*.

Fig. 14. Ova in the alveoli of S.H. 28-4. 170X. (ov - ova, pl - pleura).

Fig. 15. Ova and first-stage larvae in the alveoli of S.H. 28-3. 80X. (la - larvae, ov - ovum, pl - pleura).


pl
ov
14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period of patency. The first pattern included a rapid rise in output to a peak of 320-447 LPG of feces in 12-23 days, followed by a rapid decline to a low level at 30-40 days, then a gradual decline to the end of patency as exhibited by S.H. 21's offspring (Fig. 16a,b,c,d). The second pattern, exhibited by S.H. 28's offspring, was characterized by an erratic rise to a peak of less than 100 LPG of feces, with a steady decline thereafter (Fig. 17a,b).

No worms were recovered from four of the five hares necropsied post-patency. One male and one gravid female were recovered from S.H. 21-4. Lesions up to 3mm in diameter were found on the surfaces of the diaphragmatic lobes of all post-patent lungs; 3/5 involved both lobes, 2/5 involved the right lobe only. There was less than 1 LPG of lung tissue in S.H. 21-1, 5 LPG in S.H. 21-3, and there were no larvae in the remaining three.

No ova or larvae were found in sections of post-patent lung tissue. Lungs of each hare exhibited similar pathology. There was frequent metaplasia of bronchiolar epithelium to mucus-producing goblet cells (Fig. 18), and hyperplasia of bronchiolar epithelium (Fig. 19). Parabronchiolar lymphoid nodules were abundant, and there was extensive atelectasis (Fig. 20). Perivascular infiltration of mononuclear cells was also seen in most sections (Fig. 21).

Immature *P. boughtoni* (early L5's) were found coiled within the alveoli of the lung (Fig. 22). They were most often located immediately below the pleura, but were

Figure 16. Larvae of Protostrongylus boughtoni per gram of feces determined for experimentally-infected offspring of snowshoe hare S.H. 21 through the periods of patency.

a. S.H. 21-1.

b. S.H. 21-2.

c. S.H. 21-3.

d. S.H. 21-4.

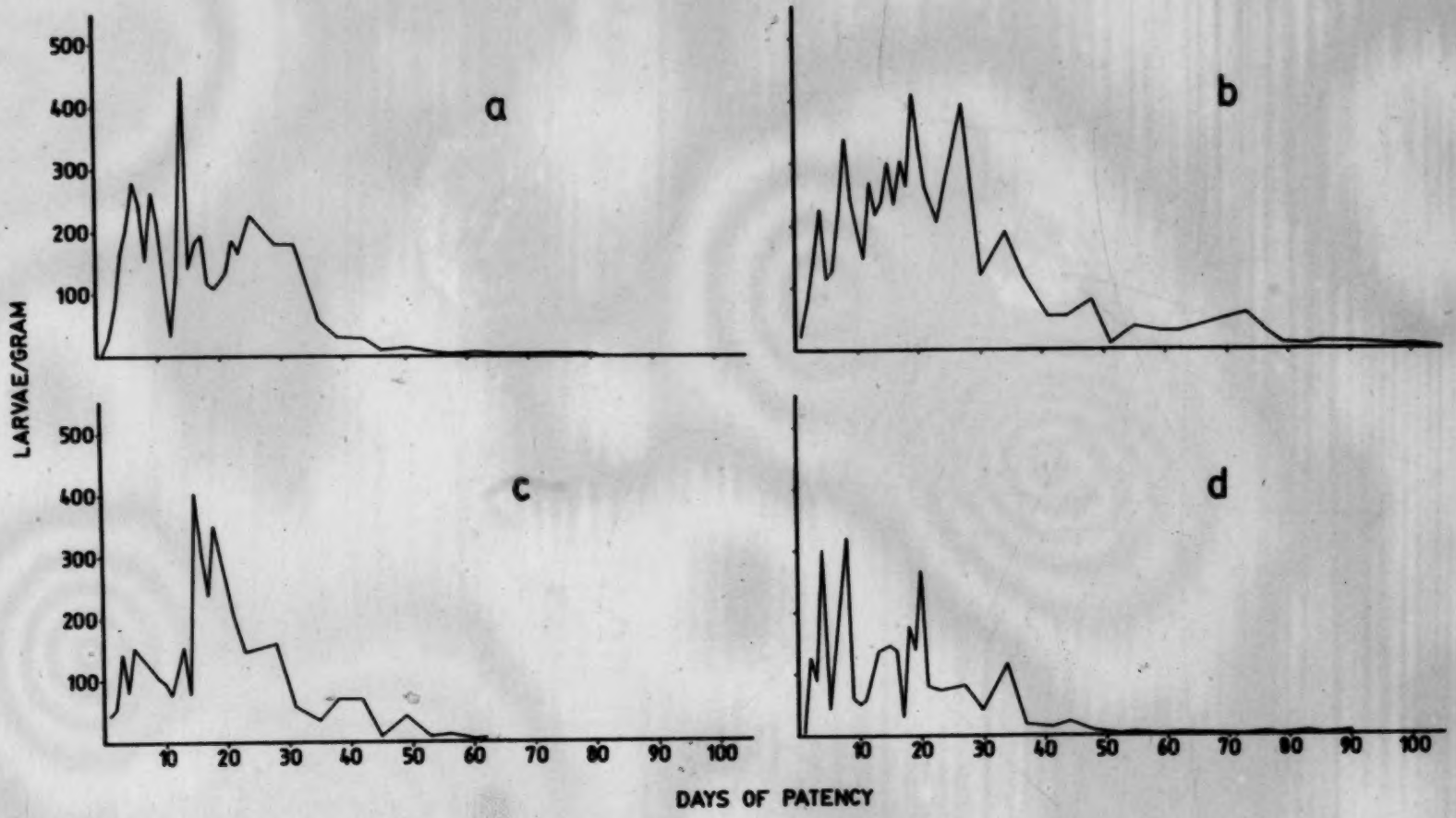
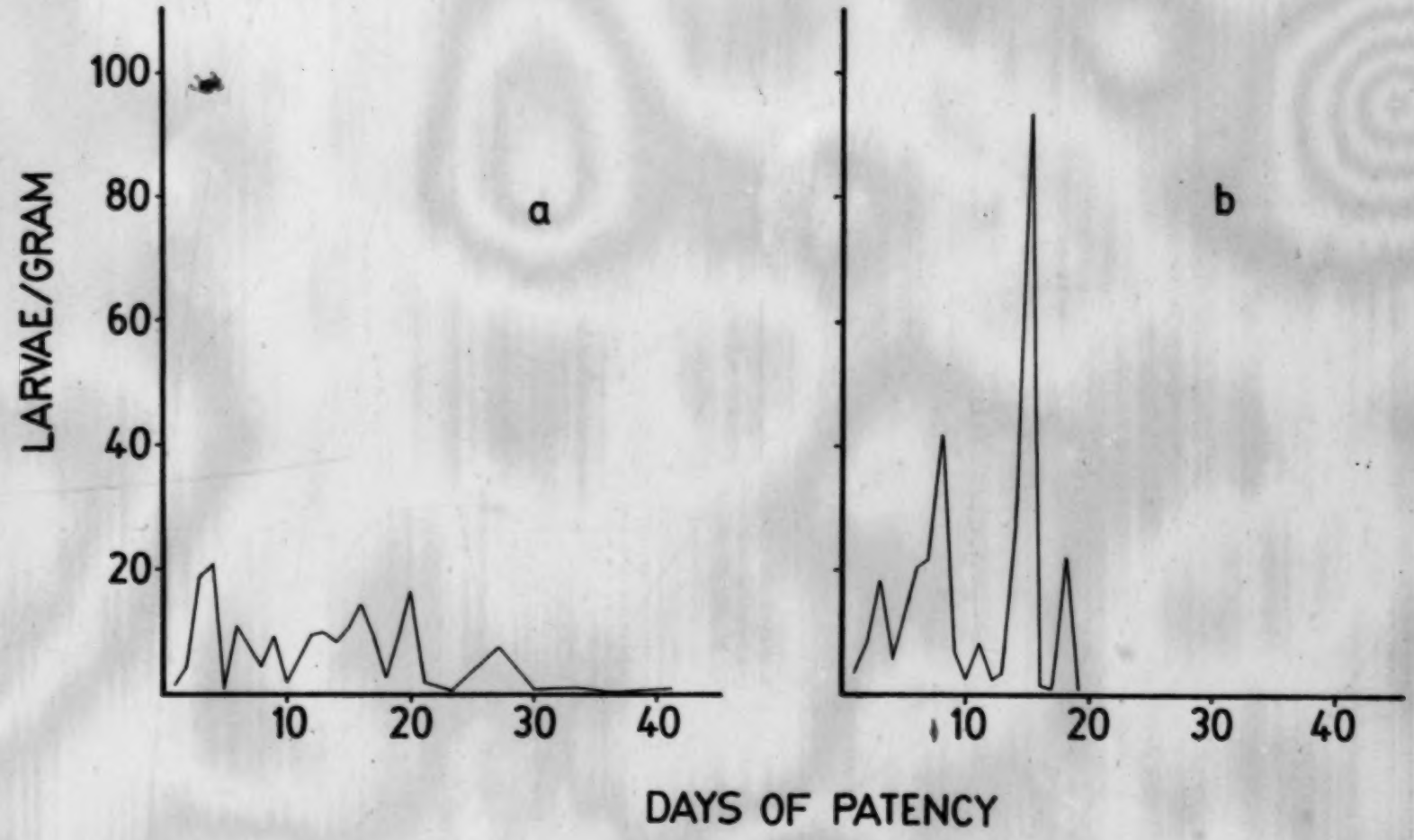

a
b
c
d
500
400
300
200
100
LARVAE/GRAM
10
20
30
40
50
60
70
80
90
100
DAYS OF PATENCY

Figure 17. Larvae of Protostrongylus boughtoni per gram of feces determined for experimentally-infected offspring of snowshoe hare S.H. 28 through the periods of patency.

a. S.H. 28-1.

b. S.H. 28-3.


LARVAE/GRAM
100
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30
40
DAYS OF PATENCY

Figures 18-21. Histological pathology of Lepus americanus lungs. Post-patent Protostrongylus boughtoni experimental infections.

Fig. 18. Metaplasia of bronchiolar epithelium to goblet cells. 300X. (gc - goblet cells, m - mucus).

Fig. 19. Hyperplasia of bronchiolar epithelium. 325X. (ef - epithelial folds).

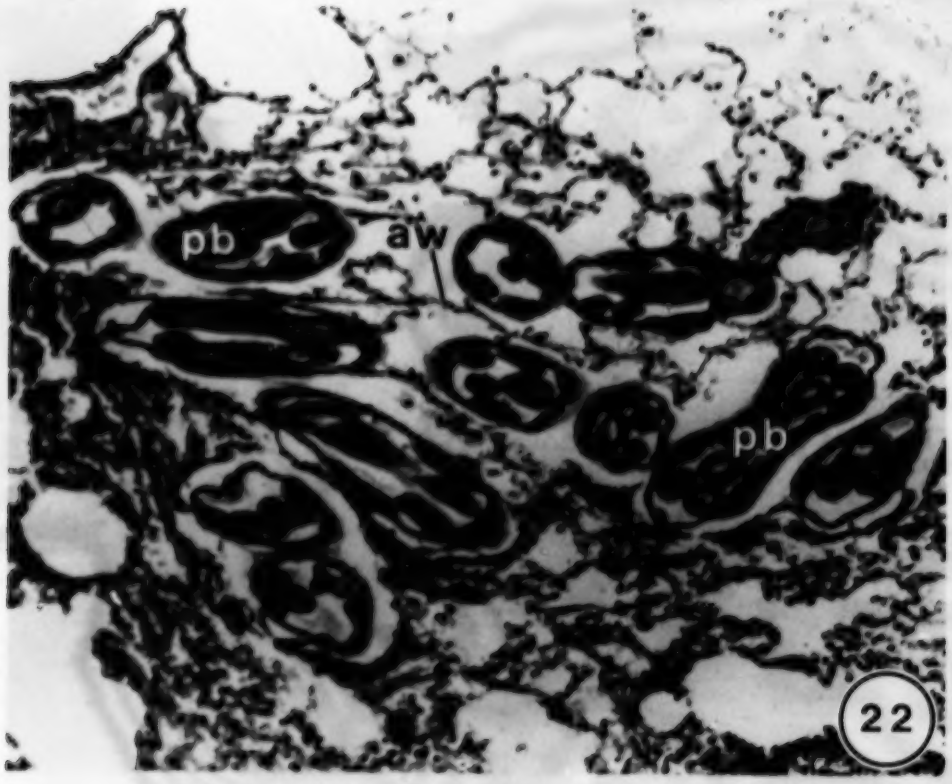
Fig. 20. Parabronchiolar lymph nodule and atelectasis. 160X. (at - atelectasis, ln - lymph nodule).

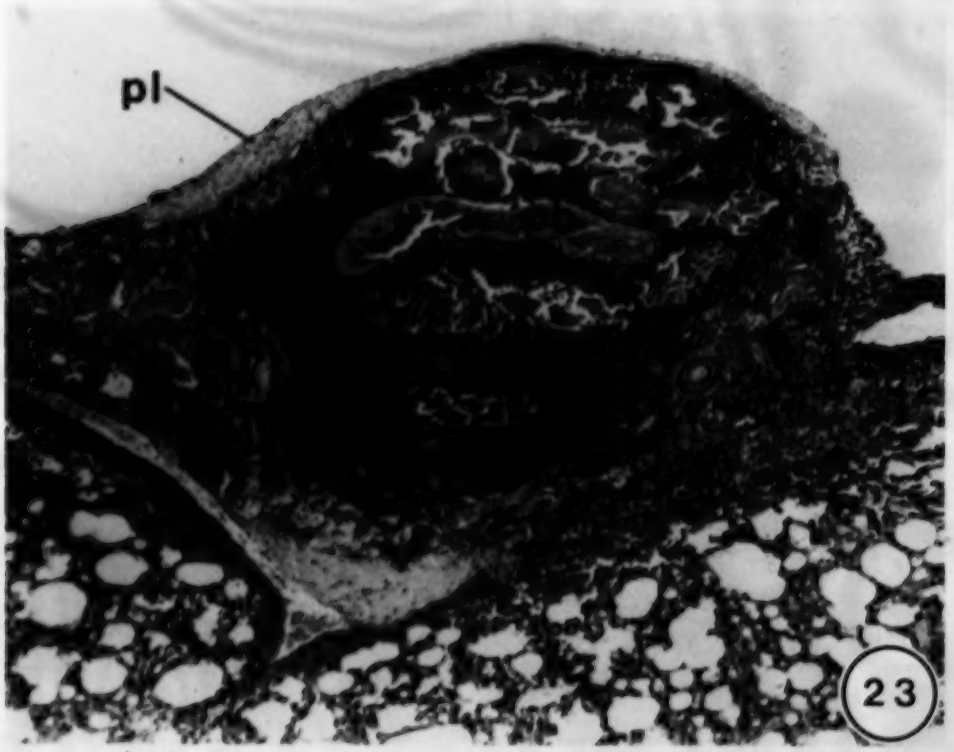
Fig. 21. Perivascular infiltration of mononuclear cells. 400X. (a - arteriole, mc - mononuclear cells).

18
gc
m
19
ef
20
ln
21
mc

Figure 22. Immature Protostrongylus boughtoni in Lepus americanus lung alveoli. 160X. (aw - alveolar wall, pb - Protostrongylus boughtoni).

Figure 23. Degenerate Protostrongylus boughtoni in a nodule at the surface of Oryctolagus cuniculus lung. 50X. (pb - Protostrongylus boughtoni, pl - pleura).


pb
aw
pb
22


pl
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sometimes found deeper within a lobe.

Experimental Infection of Rabbits

The two rabbits exposed to 50 L3's of *P. boughtoni* developed patent infections 25 and 26 d.p.e., and both patent periods lasted 13 days. Larval production was erratic and low, never exceeding 10 LPG of feces.

No live *P. boughtoni* were recovered from the post-patent lungs (both animals were terminated within one day of the end of patency), but all lobes, most frequently the diaphragmatic, were riddled with translucent and white lesions up to 3mm in diameter. Degenerate and apparently calcified worms were found in some of the larger nodules (Fig. 23).

Neither of the 2 rabbits exposed to 5,000 L1's developed patent infections. Rabbits were necropsied at 41 and 61 d.p.e. and no adult or larval *P. boughtoni* were recovered from the lungs.

The rabbit exposed to 23 L3's that had emerged from *V. pulchella* began producing L1's in the feces 28 d.p.e. The animal was immediately necropsied, resulting in the recovery of 2 male and 2 female *P. boughtoni* adults from the lungs. Many small lesions were noted, mainly on the diaphragmatic lobes, and the lungs contained 8.9 LPG of tissue.

Transplacental Transmission

Examination of 15 prenatal and post-partum young from June, 14 from July and 4 from August failed to provide evidence of *P. boughtoni* infection (Appendix II). The hares

ranged in age from 36 days post-conception to 65 days old. The mother of each individual was infected with *Protostrongylus*.

Discussion

The peroral entry of *P. boughtoni* first-stage larvae into the intermediate host would be unusual for a lungworm in the Protostrongylidae. Platt (1978) postulates that the normal mode of entry of protostrongylid larvae is via direct penetration of external gastropod epithelium with development occurring strictly in the foot. This hypothesis is supported by most work on ruminant lungworms (Hobmaier and Hobmaier 1930; Kassai 1958; Antipin 1960; Platt 1978).

Protostrongylid larvae may not be strictly excluded from peroral entry although Hobmaier and Hobmaier (1934) stated that *Protostrongylus rufescens* L1's swallowed by terrestrial snails (order Stylommatophora) perish, and Kassai (1958) extended this conclusion to all protostrongylids. Anderson (1963) frequently found first-stage larvae of *Parelaphostrongylus tenuis* about the viscera of recently-exposed snails and proposed that they would migrate into the foot. The terrestrial snails *Zonitoides arboreus* and *Vitrina limpida* commonly ingested larvae of *Parelaphostrongylus odocoilei*, but the subsequent viability of these larvae was unknown (Platt 1978).

Although the peroral mode of entry is common among carnivore lungworms in the Angiostrongylidae and Filaroididae (Platt 1978), other modes are known. Mackerras

and Sanders (1955), Cheng and Alicata (1965), Courdurier *et al.* (1967), and Yousif and Lammler (1977) showed that *Angiostrongylus cantonensis* can infect gastropods by penetration of the foot as well as through ingestion. Similar results were found for *Angiostrongylus makerrasae* (Bhaibulaya 1975), *Angiostrongylus malayensis* (Sullivan *et al.* 1978), *Aelurostrongylus pridhami* and *Filaroides martis* (Anderson 1962). Direct penetration of the gastropod foot was the only mode of entry reported for *Aelurostrongylus abstrusus* (Hobmaier and Hobmaier 1935) and *Anafilaroides (=Oslerus) rostratus* (Klewer 1958; Seneviratna 1959).

In the present study, the absence of first-stage larvae of *P. boughtoni* in the foot of *V. pulchella* after a standard period of exposure (2 1/2 hrs.) indicates that infection may have occurred via the peroral route. At some later period, the larvae might penetrate the wall of the alimentary canal and migrate to the foot of the snail, as developing larvae were never found in other areas of the body.

Platt (1978) stated that the method of exposing molluscs to metastrongyloid larvae may affect the primacy of one mode of entry over the other. Specifically, snails exposed to larvae in water were infected perorally, while those exposed to larvae on filter paper were infected by direct penetration. On this basis, direct penetration of *V. pulchella* by *P. boughtoni* L1's was predicted but not observed. Kadenatsii (1969), however, reported that first-stage larvae of *Protostrongylus tauricus*, a lungworm

of *Lepus europaeus*, penetrated the foot of *Vallonia costata* and *Vallonia enniensis* "by means of active drilling movements". Thus, it is possible that the mode of entry of metastrongyloid larvae into molluscs is a species character and not common to a nematode family, among lungworms of a particular mammalian order, within molluscan orders, or to a particular exposure technique. Also, some species may not be restricted to a single mode of entry.

Reports of mode of entry are no doubt greatly influenced by a researcher's methods. For example, Morera (1973) exposed slugs to *A. costaricensis* by coating lettuce with feces from infected rats, and concluded that larvae entered the slugs by ingestion even though all larvae were found in the foot and mantle, suggesting entry by direct penetration. Anderson (1962) points out that after Hobmaier and Hobmaier's (1930) histological discovery of *Protostrongylus rufescens* L1's penetrating the foot of snails, many subsequent authors stated other species of larvae used this mode of entry without providing evidence. In light of all the factors mentioned, a great deal of re-investigation would be necessary to draw any conclusions concerning the prime mode of entry of groups within the Metastrongyloidea.

Development of *P. boughtoni* in *V. pulchella* was similar to that of certain other metastrongyloids in their intermediate hosts. A period of almost no increase in length throughout the first-stage was followed by a period of rapid

elongation in the second-stage. This pattern of growth has been reported for *P. odocoilei* (Platt 1978), *Elaphostrongylus rangiferi* (Halvorsen and Skorping 1982), *Troglostrongylus brevior* (Gerichter 1949), *A. costaricensis* (Morera 1973) and in one study, *A. cantonensis* (Rachford 1976).

More commonly seen in this group of nematodes is a pattern of rapid increase in length in the first-stage, followed by decreased growth in the second and third. One variation within this pattern is a five to seven day period of constant length post-exposure, followed by greatly accelerated growth until the first molt (Gerichter 1951; MacKerras and Sanders 1955; Seneviratna 1959). The second variation is constant growth in length throughout the first-stage, followed by decreased growth in the second and third-stages (Gerichter 1949,1951; Beresford-Jones 1966; Drodz *et al.* 1971; Bhaibulaya 1975). Of the *Protostrongylus* species for which there are published measurements of all larval stages, *P. stilesi*, *P. rufescens* and *P. tauricus* all exhibit greatest growth in length before the first molt (Gerichter 1951; Pillmore 1956; Kadenatsii 1969), unlike *P. boughtoni*.

Many authors report growth in width of developing metastrongyloid larvae during the first-stage (Hobmaier and Hobmaier 1935; Gerichter 1949; MacKerras and Sanders 1955; Pillmore 1956; Seneviratna 1959; Rachford 1976; Platt 1978), a characteristic absent in the present study. MacKerras and

Sanders (1955) stated that the first-stage of *A. cantonensis* was the only feeding stage and the observed increase in width was due to storage of refractile food granules in the intestine. In the present study, the width of *P. boughtoni* larvae did not increase until the second-stage, and refractile granules were not apparent until this period, indicating that most feeding occurs after the first molt. The general decrease in width observed after the second molt is not uncommon (Pillmore 1956; Morera 1973; Rachford 1976).

Development of metastrongyloid larvae is reported from a wide variety of aquatic and terrestrial snails and no doubt there is host-induced variation in the patterns observed. Mackerras and Sanders (1955) reported "considerable" growth in length of *A. cantonensis* in the terrestrial slug *Agriolimax laevis* prior to the first molt. Rachford (1976), in contrast, found growth in length of the same parasite during the second and third-stages only in the aquatic snail *Lymnaea palustris*. It is thus difficult to demonstrate affinities of *P. boughtoni* to other *Protostrongylus* species based on growth patterns.

Although a pre-infective period of the third-stage (Gerichter 1948) was not determined for *P. boughtoni*, it could possibly exist immediately after the second molt and before the outer first-stage cuticle darkens, a period of less than three days. However, Gerichter (1951) determined there was no pre-infective third stage in *P. rufescens* and *P. boughtoni* L3's with darkened cuticles were found in the

present study to be infective.

The general increase in variability of lengths of *P. boughtoni* larvae as development proceeded could have several causes aside from inherent genetic expression of the larvae and snails. Development of *Parelaphostrongylus tenuis* is dependent on the activity of the gastropod host (Lankester and Anderson 1968). Zmoray and Svarc (1960) and Cabaret (1981) found that development of protostrongylid larvae varied with the age of the intermediate host. Also, there may be an undetermined "crowding effect" (Read 1951) in heavily-infected snails, resulting in decreased growth.

Comparison of development time of protostrongylids to the infective stage with literature values is difficult due to the influence of different species of molluscs (Kassai 1958; Skorping and Halvorsen 1980) and temperatures at which infected molluscs are kept (Gerichter 1948,1951; Rose 1957; Halvorsen and Skorping 1982).

The pre-infective periods of eight other larval *Protostrongylus* species, plus one morphologically and biologically similar *Orthostrongylus* species, are presented in Table V. Most researchers document development of *Protostrongylus* in snails at "room temperature" (20-25°C). If this procedure had been followed in the present study, the resultant pre-infective period would have been shorter, but still well within the range of other *Protostrongylus* species.

TABLE V. COMPARISON OF DURATION OF LIFE CYCLE PERIODS AMONG LEPORID *PROTOSTRONGYLUS* SPECIES AND RELATED LUNGWORMS OF RUMINANTS

SPECIES (SOURCE)	INTERMEDIATE HOST	PREINFECTIVE PERIOD	DEFINITIVE HOST	PREPATENT PERIOD	PATENT PERIOD
P. boughtoni (This Study)	*V. pulchella*	27-30d	*Lepus americanus*	25-27d	41-104d
P. cuniculorum (Joyeux and Gaud 1946)	*Helicella* spp.	-	*Oryctolagus cuniculus*	26-37d	-
P. kamenskyi + *P. pulmonalis* (Rizhikov *et al.* 1956a,b)	*Vallonia tenuilabris*	30-35d	*Lepus variabilis*	19-20d	-
P. sylvilagi (Pillmore 1959)	*Pupilla blandi*, *V. pulchella*	-	*Sylvilagus auduboni*	35-54d	17-25d
P. tauricus (Kadenatsii 1969)	*Vallonia*, *Helicella* spp.	20-25d	*Lepus europaeus*	25-32d	8-9mo
P. hobmaieri (Politov 1973)	23 sp. & subsp. land gastropods	16-39d	-	-	-
P. raillieti (Ubaidullaev 1975)	*Helicella candaharica*	26-30d	Domestic sheep + goats	35d	-

TABLE V. CONTINUED

SPECIES (SOURCE)	INTERMEDIATE HOST	PREINFECTIVE PERIOD	DEFINITIVE HOST	PREPATENT PERIOD	PATENT PERIOD
P. rufescens (Kassai 1962; Kulmamatov 1977)	H. candaharica, Subzebrinus albiplicatus	19-44d	Domestic sheep + goats	34-37d	28mo +
P. skrjabini (Azimov et al. 1976)	H. candiharica, S. albiplicatus	25-30d	Domestic sheep + goats	30d	-
P. stilesi (Monson and Post 1972)	V. pulchella	14d	Bighorn/mouflon hybrid	63-122d	-
P. stilesi and/or P. rushi (Pillmore 1956, 1959; Lange 1973)	P. blandi, V. pulchella	11-60d	Bighorn/domestic hybrid	60d	60d
			Bighorn	42-56d	9-15mo +
O. macrotis (Pillmore 1959; This Study)	V. pulchella	40d	Odocoileus hemionus	40-54d	-

The exit of third-stage larvae from the bodies of infected gastropods has been reported for species in the Angiostrongylidae and Protostrongylidae. Cheng and Alicata (1964) first noted the emergence of infective *A. cantonensis* L3's from two species of terrestrial snail and one species of slug partially submerged in water. Heyneman and Lim (1967) calculated that small terrestrial slugs in Malaya containing high numbers of *A. cantonensis* spontaneously passed infective larvae at a rate of 0.25 L3/slug/24hrs. Richards and Merritt (1967) reported the emergence of larvae of the same species from the aquatic snail *Biomphalaria glabrata* after crushing. More recently, third-stage larvae of *A. costaricensis* were found in the mucus secretion of infected slugs (Morera 1973) and emerged from *B. glabrata* into water (Ubelaker *et al.* 1980).

Within the Protostrongylidae, emergence of third-stage *Protostrongylus* larvae from snails has been reported most frequently by Russian authors (Davtyan 1947; Antipin 1960; Fedorov 1960; Kadenatsii 1969) who contend that infection of the definitive host occurs primarily through ingestion of isolated larvae on vegetation and in water, although once in the external environment the period of viability of such larvae is unknown. Naumov (1947) observed that infective larvae of protostrongylids were found on vegetation in places where hares fed. High densities of larvae in the environment were held accountable for the high prevalence and intensities of protostrongylosis in hares in Crimea

(Kadenatsii 1969). In other reports, Monson and Post (1972) noted third-stage larvae of *P. stilesi* leaving the foot of *V. pulchella* and Rose (1957) and Zmoray *et al.* (1969) reported L3's of *Muellerius capillaris* leaving decomposing gastropods. In the present study, at least some (4/23) of the emerging L3's proved to be infective, lending a measure of support to the Russian hypothesis. However, these larvae were not subjected to extreme environmental conditions of heat and desiccation.

The Russian authors claim that infective larvae actively crawl out of the snail foot. *P. boughtoni* L3's often were observed twisting and coiling within the snail tissue, and in two instances L3's migrated from the foot to the viscera. *A. cantonensis* apparently emerges without extraneous cuticles from the first two molts, and L3's have been observed migrating through snail tissue without an encapsulating cyst (Harris and Cheng 1975; Sullivan *et al.* 1978).

A possible route of exit of *Protostrongylus* larvae from the snail, aside from active crawling, is passive expulsion of the larva cyst by an immune mechanism. Cheng and Rifkin (1970) note that an immune response common to all molluscs is phagocytization of small particles by amoebocytes followed by the transportation of these through epithelial surfaces to the outside. Large particles usually elicit an initial amoebocytic encapsulation response reinforced by fibroblastic nodule formation. The mechanism may exist which

would allow transport of such large cysts across the outer epithelial layers in the same manner as phagocytic amoebocytes. Evidence for this in the present study was the observation that infective larvae did not escape from dead snails.

The survival of third-stage metastrongyloid larvae in the environment is no doubt shorter than that for most first-stage larvae. *A. cantonensis* L3's may survive up to 72 hours in water (Cheng and Alicata 1964; Richards and Merritt 1967). Heyneman and Lim (1967) demonstrated that *A. cantonensis* L3's collected from lettuce bought in a Kuala Lumpur market were infective. L3's of *Muellerius capillaris* remain infective for at least six months at -12°C (Sauerlander 1979). Larval survival is likely contingent on continuous hydration, as *A. cantonensis* L3's survive drying for only a few minutes (Richards and Merritt 1967). Intermediate hosts of *Protostrongylus* are usually snails of dry environments (Gerichter 1951; Pillmore 1956) and this could limit the impact of L3 transmission via emergence onto vegetation. Considering these physical restrictions, the importance of larval emergence in transmission of *P. boughtoni* can only be a matter for speculation.

The emergence of infective larvae of some parasites may occur only when gastropods are heavily infected. Techniques for exposing snails to *A. cantonensis* may result in infections of over 2,000 larvae (Richards and Merritt 1967). Digestion of snails exposed to *A. costaricensis* yielded from

850 to 2,500 larvae each (Ubelaker *et al.* 1980) and levels of natural infections of the same species in Malaya, where larval emergence has been reported, averaged 250 larvae per snail (Heyneman and Lim 1967). In the present study, snails were infected with 3-17 larvae, while the mean intensity in natural infections was less than 2 (Chapter II). In both Kadenatsii's (1969) and the present study, it was observed that many snails did not become totally free of infection. Emergence of infective larvae may be limited to unusually heavy infections and thus rare in naturally-infected intermediate hosts of *P. boughtoni*.

Pillmore's (1956) measurements of *P. boughtoni* L1's fall within the ranges derived in this study, although the widths differ, possibly due to different coverslip pressures on the worms. Lengths of L1's reported by Goble and Dougherty (1943) are greater, perhaps as a result of using a fixative and clearing agent other than glycerin-alcohol/glycerin which could produce a stretched cuticle (Fagerholm 1979). *P. boughtoni* L1's are generally smaller than those of other leporid *Protostrongylus* species with the exception of *P. pulmonalis*.

Of the other wild-mammal species of first-stage *Protostrongylus*-type larvae that may be encountered within the distribution of snowshoe hares, neither *Orthostrongylus macrotis* of mule deer or *P. stilesi* of bighorn sheep can presently be differentiated on the basis of morphological characteristics. Measurements of *P. rushi* larvae, however,

all fall outside the upper range for *P. boughtoni* and, thus, could be differentiated. Pillmore (1956) suggested that characters such as tail length, movement and color could also be used to separate L1's of different protostrongylid species.

The advanced second-stage larva of *P. boughtoni* is generally larger, but similar morphologically to the L1. Phasmids, unobserved on the L1, are visible, and lateral alae are absent. The intestine of second-stage larvae of metastrongyloids in the snail host is commonly filled with refractile granules, likely food reserves.

Infective larvae of *P. boughtoni* are smaller than leporid L3's for which there are published measurements. Infective larvae of lungworms from bighorn sheep, mule deer and hares have the same superficial appearance in their common snail intermediate hosts. It would be of value if infective larvae could be identified as to source mammal by length in areas where these three animals were sympatric. The maximum length of *P. boughtoni* falls below the range of that for sheep *Protostrongylus* spp. and these larvae are separable on this basis. *P. boughtoni* overlaps with *O. macrotis*, although the two groups are statistically different (Mann-Whitney $U=2$, $p<0.001$). In the same manner, *O. macrotis* overlaps with sheep *Protostrongylus* spp. in spite of statistical difference ($U=85$, $p<0.01$). Therefore, in areas where only snowshoe hares and bighorn sheep were sympatric, the species of infective *Protostrongylus*-type

larvae could be determined with confidence.

Although there is no development of the terminal genital apparatus in the fourth-stage, the genital primordium is greatly enlarged anteriorly and posteriorly. The amount of enlargement was quite variable, indicating continual growth throughout this stage. Dark brown, ingested material in the intestine suggests that the L4 feeds on blood in the lungs. Since the fourth-stage of *P. boughtoni* is found in the lungs, the third-stage may migrate through the definitive host as is the case with *Parelaphostrongylus tenuis* (Anderson and Strelive 1967) and *Protostrongylus taurlcus* (Kadenatsii 1969).

Two of the major factors which hinder the establishment of a parasite within an abnormal host are: 1) absence of specific environmental conditions necessary for growth and development; and, 2) resistance of the host (Noble and Noble 1976). Either factor acting upon an established parasite could delay development, inhibit growth, or destroy it outright. Often it is not possible to separate immunological from physiological incompatibility (Lackie 1980).

The immune response (encapsulation, phagocytization) to parasites within compatible molluscs is reduced as a general rule (Malek and Cheng 1974). Sullivan and Richards (1981) reported that only non-susceptible strains of *B. glabrata* produced a significant encapsulation response to *Schistosoma mansoni* sporocysts. Drodz *et al.* (1971) demonstrated responses ranging from massive to no encapsulation when 17

species of terrestrial and aquatic molluscs were infected with *A. cantonensis*. The same parasite in *Marisa cornuarietis*, an unsuitable host, provoked a severe encapsulation response, as well as a generalized proliferative reaction in the vascular and connective tissue (Yousif *et al*. 1980).

The factors which influence the compatability of larval parasites with intermediate hosts appear to have a genetic base (Fransden 1979). The intensity of host response is probably related to the degree to which the mollusc recognizes the parasite as "nonself" (Malek and Cheng 1974). There is evidence that larval stages of some parasites utilize antigen masking to evade the immune response of an invertebrate host (Lackie 1980). Only through an extended period of natural selection would such a defense arise (Damian 1979).

The histologically-observable immune response of *V. pulchella* to third-stage larvae of *P. boughtoni* was indistinguishable from that of *V. gouldi*, the major intermediate host in the Slave Lake study area (Chapter II).The encapsulation response in both cases was slight, indicating that there was not sufficient recognition of the larva by either snail as nonself to result in a more severe immune response.

The growth and rate of development of protostrongylid larvae can vary widely depending on the species of intermediate host (Skorping and Halvorsen 1980; Urban 1980).

In the present study, the body length achieved by *P. boughtoni* in *V. pulchella* was indistinguishable from that in *V. gouldi*. Rates of development of larvae in *V. pulchella* and *V. gracilicosta*, a local intermediate host of *P. boughtoni*, were also similar.

On the basis of these three criteria, *V. pulchella* appears to be a highly suitable intermediate host for *P. boughtoni*, and measurements of larvae taken from this snail can be used in taxonomic descriptions. Although outwardly dissimilar in appearance, the Valloniidae are very closely related to the Pupillidae (Pilsbry 1948), both likely descendants of the same pupillid stock. Physiological similarity may enable *P. boughtoni* to develop equally well in species of either family. In addition, although *V. pulchella* is not native to western North America it is indigenous to the east, being recorded in Pleistocene deposits (Pilsbry 1948). No doubt *V. pulchella* is a natural intermediate host in the eastern range of *P. boughtoni* where it occurs in snowshoe hare habitat.

The route of migration through the definitive host was not determined for *P. boughtoni*. Antipin (1960) reported that ruminant *Protostrongylus* larvae penetrate the intestinal wall, enter the circulatory system and move to the mesenteric lymph nodes. From there they travel to the lungs and pass into the lumina of the organ. Third-stage larvae of *P. tauricus* were found in the mesenteric lymph nodes of rabbits and hares 24-36 hours post-exposure, and in

the liver and lungs after 4-6 days (Kadenatsii 1969).

The prepatent period of 25-27 days for *P. boughtoni* in the snowshoe hare is similar to that of many other species of *Protostrongylus* in their normal definitive hosts (Table V). Since there are no reports of natural infections of Audubon's cottontail with *P. sylvilagi*, the slightly longer than average prepatent period in this experimental host may be the result of suboptimal compatability. Also, the longer prepatent periods of the bighorn sheep lungworm infections may be attributed to unnatural definitive hosts in the first two instances (Monson and Post 1972; Pillmore 1956,1959, respectively), and some measure of concomitant immunity in the third (Lange 1973).

Since the length of the prepatent period may vary inversely with the number of infective larvae administered (Platt 1978), it is difficult to make comparisons among *Protostrongylus* species as authors either neglect to report the number of larvae, or the numbers are widely variant. Most *Protostrongylus* species have prepatent periods of less than 50 days, and *P. boughtoni* at 25-27 days is in approximately the middle range of reports. The narrow range of prepatent periods achieved in the present study is likely due to the use of experimental animals never previously exposed to helminth infection, a procedure not often followed in *Protostrongylus* research.

The pattern of L1 numbers in the feces of S.H. 28's offspring was extremely low and erratic, indicating the

possibility of an inherited resistance to *P. boughtoni* infection. The pattern of S.H. 21's offspring was more typical of protostrongylid infections, but with some differences. Although the initial, rapid rise in larval output is common to protostrongylids (Panin 1964; Nettles and Prestwood 1976; Platt and Samuel 1978), there was no comparable plateau phase extending over a long period of time. Instead, there was a steady drop to a low level at 30-40 days, and the output remained very low until the end of patency.

The patent periods of five other *Protostrongylus* species are listed in Table V. The period of larval output for *Protostrongylus* in leporids is generally shorter than that in ruminants, and could be an adaptation to the relatively short life span of the definitive host. Reinfection may extend the period of larval output if concomitant immunity does not block re-establishment of the parasite.

The pathological changes in the lung induced by *P. boughtoni* are commonly seen with many species of metastrongyloids (Stockdale, 1976). Green and Shillinger (1935a), and Green *et al.* (1939) reported similar lesions from snowshoe hares naturally infected with *P. boughtoni*, plus necrosis and some pleural adhesions. These lesions were also common to *Lepus timidus* with mixed infections of *P. kamenskyi* and *P. pulmonalis* (Tsvetaeva and Mozgovoi 1956), *Lepus europaeus* with infections of *P. pulmonalis* and *P.*

taurlcus (Babos 1961), and to *Oryctolagus cunlculus* with *P. taurlcus* and *P. oryctolagl* (Babos 1961).

Few researchers have examined development of *Protostrongylus* species in the definitive host. It is possible that *P. boughtonl* migrates to the lung through the circulatory system and breaks out of a capillary into an alveolus, or through the coelom, across the diaphragm and then penetrates the pleura. As it continues to molt and grow, it likely pushes through septa and winds through many alveoli. At some time before maturity, it must move from this environment into a bronchiole to complete its life cycle. Third- or fourth-stage larvae may be incapable of holding their position against the ciliary motion in the bronchioles, and the parasite only moves into that environment when large enough to resist the efforts to evict it.

The shortened patent period, combined with very low larval output, indicates that *O. cunlculus* is a poor definitive host for *P. boughtonl*. The immune response of this species appears capable of destroying adult worms before a significant number of larvae are produced. This is evidenced in the calcified remnants of worms found in the numerous nodules throughout the lungs; a common immune reaction to incompatible parasites as an analogous response was reported to *Dirofllarla lmmltis* in the lung of a laboratory rabbit (Narama *et al.* 1982). Similar calcified nodules were found by Pillmore (1959) in the lungs of a New

Zealand white rabbit exposed to *P. sylvilagi*. In later research, he managed to produce a patent infection in this strain by intravenous injection of L3's (Pillmore 1961). The prepatent period in this case was 21 days. Kadenatsii (1969) reported that rabbits exposed to *P. tauricus* became patent at the same time as hares exposed similarly, but the patent period was 3-5 months as opposed to 8-9 months. Rizhikov *et al.* (1956b), exposing rabbits to a combination of *P. kamenskyi* and *P. pulmonalis*, determined that the prepatent period was extended to 22 days in contrast to 19-20 days for infections in hares (Rizhikov *et al.* 1956a).

It appears that leporid *Protostrongylus* species in abnormal hosts exhibit normal or extended prepatent periods, shortened patent periods, and induce more severe pathology. Further evidence is provided in a report of *P. boughtoni* infections by Goble and Cheatum (1944) that, "more severe pathological conditions in the rabbits (*Sylvilagus floridanus mallurus*) than those observed in hares also supports the view that the former is an abnormal host". Since the life cycle of *P. boughtoni* was completed in *O. cuniculus*, however, this species may be useful as a laboratory host in investigations of the route of larval migration, and of alternate modes of transmission.

Exposure of two *O. cuniculus* to first-stage larvae of *P. boughtoni* did not result in establishment of an infection after more than twice the normal prepatent period. The failure of this exposure may be due to the use of an

abnormal host as well as to the absence of this mode of transmission in the normal life cycle. Direct transmission could be a beneficial strategy for a leporid parasite, considering that members of this family are obligatorily coprophagic. However, direct transmission has only been recorded for metastrongyloids of carnivores (Angiostrongylidae and Filaroididae). Dunsmore and Spratt (1979) believe that direct transmission of *Oslerus osleri* through coprophagy is not as important as through regurgitative feeding at the time of weaning of young Canidae. This mode of transmission may have evolved exclusively among regurgitative feeders in the Carnivora as there are no reliable reports of direct transmission in the Protostrongylidae.

CHAPTER II. TEMPORAL DYNAMICS OF PROTOSTRONGYLUS BOUGHTONI IN NATURAL POPULATIONS OF INTERMEDIATE AND DEFINITIVE HOSTS

Introduction

Most field studies on members of the Metastrongyloidea report prevalence and intensity of infection of hosts as instantaneous values, although infection rates can be seasonally variable. Such variability may be critical for transmission of the parasite, especially if there are seasonal changes in susceptibility of intermediate (Cabaret 1981) and definitive (Kontrimavichus and Popov 1960) hosts to infection. Although the lungworm *Protostrongylus boughtoni* has often been reported from the snowshoe hare (Boughton 1932; Green and Shillinger 1935b; Goble and Dougherty 1943; Goble and Cheatum 1944; Olsen 1954; Bookhout 1971), nothing is known about infrapopulation (Esch *et al.* 1975) dynamics in free-ranging definitive hosts. Similar information is lacking for the natural intermediate hosts of *P. boughtoni*, which have remained unidentified until the present study. In this chapter, I will address these aspects of the host-parasite relationship.

Studies on natural populations of intermediate and definitive hosts of the Protostrongylidae are relatively scarce. Samuel (1978) examined the seasonal infection of gastropod species with *Parelaphostrongylus odocoilei* in Jasper National Park while monitoring larval output from mule deer to determine timing and location of transmission. Lankester and Anderson (1968) documented seasonal aspects of

Parelaphostrongylus tenuis infection in gastropods on Navy Island in southern Ontario. The temporal output of first-stage larvae of *Protostrongylus stilesi* and/or *Protostrongylus rushi* from Rocky Mountain bighorn sheep was recorded by several researchers (Pillmore 1961; Forrester and Senger 1964; Uhazy *et al.* 1973; Gates 1975). In a unique study, Kontrimavichus and Popov (1960) determined the monthly prevalence and intensity of infection of *Protostrongylus pulmonalis* and/or *Protostrongylus kamenskyi* in *Lepus timidus*. The paucity of similar definitive host studies may be a reflection of the difficulty in obtaining reasonable sample sizes, and the time involved in conducting thorough necropsies.

P. boughtoni infections in *Lepus americanus americanus* and gastropod intermediate hosts were examined monthly in an area of boreal forest near Slave Lake, Alberta throughout 1981. Preliminary investigations undertaken the previous year indicated that most hares were infected, often with relatively high intensities. Also, the local hare population was at or near the peak of its cycle of abundance and, thus, individuals were easily collected for study. These factors were expected to optimize the chances of determining intermediate host species, documenting infections in intermediate hosts and all age groups of definitive hosts, and determining types of habitat in which transmission of the parasite was likely to occur.

The objectives of this portion of the study were to: 1) identify natural gastropod intermediate hosts of *P. boughtoni* and determine monthly prevalences and intensities of infection in defined habitats; 2) determine the monthly abundances of gastropod intermediate hosts in habitats; 3) monitor monthly infections of all age groups of hares with *P. boughtoni* and, in particular, document recruitment of lungworm into litter groups; and 4) determine monthly use by hares of defined habitats.

Materials and Methods

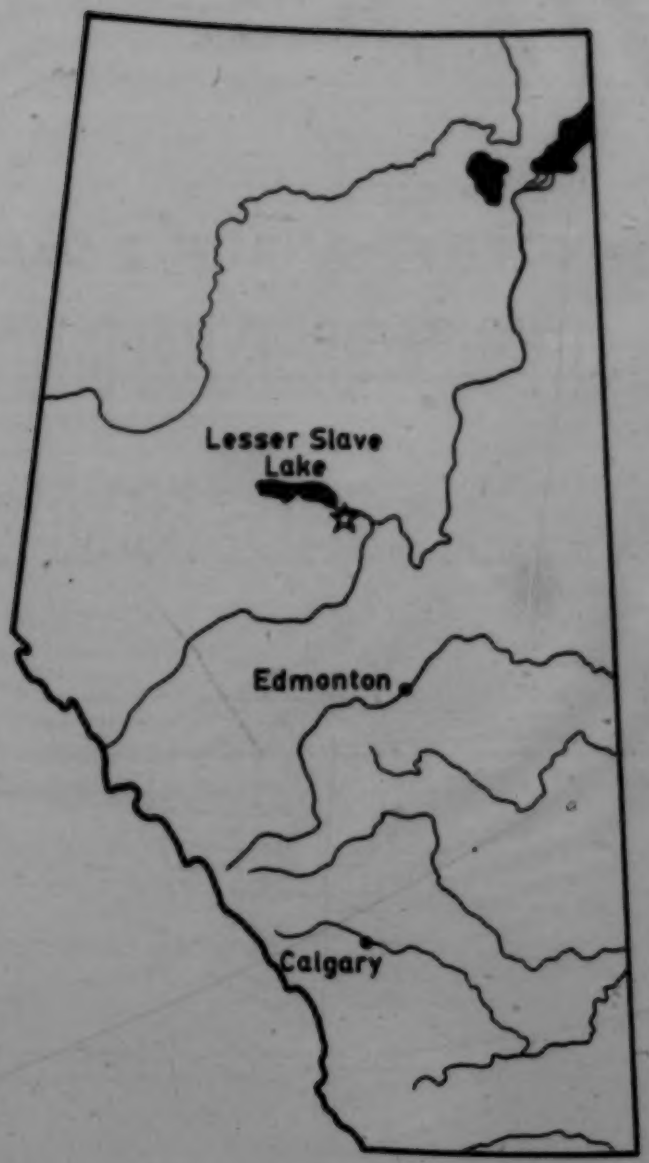
Study Area

General Description

The study area was located on the Mitsue Oil Field (Chevron Standard Ltd.) near Slave Lake, Alberta (55° 00′ to 55° 10′ N and 114° 25′ to 114° 35′ W) within the Lower Foothills region of the boreal forest (Rowe 1972) (Fig. 24). The area was characterized by rolling topography (elevation 701-762m) and small, discrete forest stands or habitat types. Sections of the area had been logged between 5 and 10 years previously with subsequent regrowth. The entire study area was dissected by a network of gravel roads and cutlines developed for the exploration and removal of gas and oil. These tracks provided access to most of the habitat types within the study area.

No official detailed classification of the forest stands exists. However, Bondrup-Nielsen (1978) determined the relative abundance of 12 designated habitat types within

Figure 24. Location of the study area in Alberta (star).


Lesser Slave
Lake
Edmonton
Calgary

the study area. These habitat types were grouped and reduced to five general types in the present study. A pictoral profile description of these five types is presented in Figure 25.

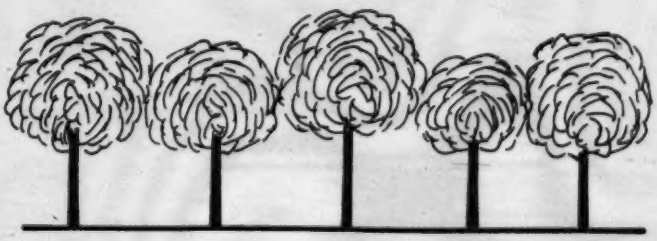
The first habitat type was designated as Stag (ST). The most abundant species of tree in this habitat was black spruce (*Picea mariana*) with the occasional occurrence of several other species of conifers and hardwoods; the canopy varied between open and closed. The woody undergrowth usually consisted of dense patches of Labrador tea (*Ledum groenlandicum*). Locations were generally on low ground; the forest floor was most often mossy and wet.

The second habitat type, Spruce Slope (SS), was dominated by mature white spruce (*Picea glauca*) or balsam fir (*Abies balsamea*) which produced a closed canopy. Understory vegetation was very sparse and of mixed composition. Locations were on high ground; the floor was dry and composed mainly of needle litter.

The third and most abundant habitat type was designated Mixed Wood (MW) and was composed of mixed coniferous and deciduous trees of all ages which produced a closed canopy. The understory was sparse and of mixed composition. Elevation and moisture varied, as did the composition of the litter floor.

The fourth habitat type, Cut (CUT), consisted of areas previously disturbed. Most of the original mature tree species had been removed, with the consequent destruction of

Figure 25. Pictorial profiles of the five designated habitat types and the estimated proportion of the study area which each occupies.



HABITAT TYPE	% OF AREA
STAG	24.0
SPRUCE SLOPE	11.7
MIXED WOOD	35.2
CUT	8.0
HARDWOOD	20.3
TOTAL	99.2

understory vegetation and stripping or redistribution of topsoil by earth- and tree-moving machinery. At the time of the present study, these areas were covered primarily with young, coniferous and deciduous regrowth from the succeeding 5 to 10 years. There were scattered, relict, mature, coniferous and deciduous trees and the canopy varied from open to closed depending on the extent of regrowth. The woody undergrowth was often very dense, the most common species being bracted honeysuckle (*Lonicera involucrata*). Elevation varied, but the forest floor was generally dry and covered very thinly with litter.

The fifth habitat type, designated Hardwood (HW), was dominated by mature aspen (*Populus tremuloides*) or balsam poplar (*Populus balsamifera*). The canopy was closed, and the most common woody undergrowth was highbush cranberry (*Viburnum trilobum*). Elevation and moisture varied, and the forest floor was covered very thickly with leaf litter.

These five groups encompassed approximately 99% of the habitat types present on the study area. Not sampled in the present study, and excluded from the groups, were thickets of roadside alder (*Alnus crispa*). Preliminary sampling indicated that, although these areas were often frequented by snowshoe hares, the substrate was depauperate of snails due to a thin layer of litter and repeated disturbance from road-grading equipment.

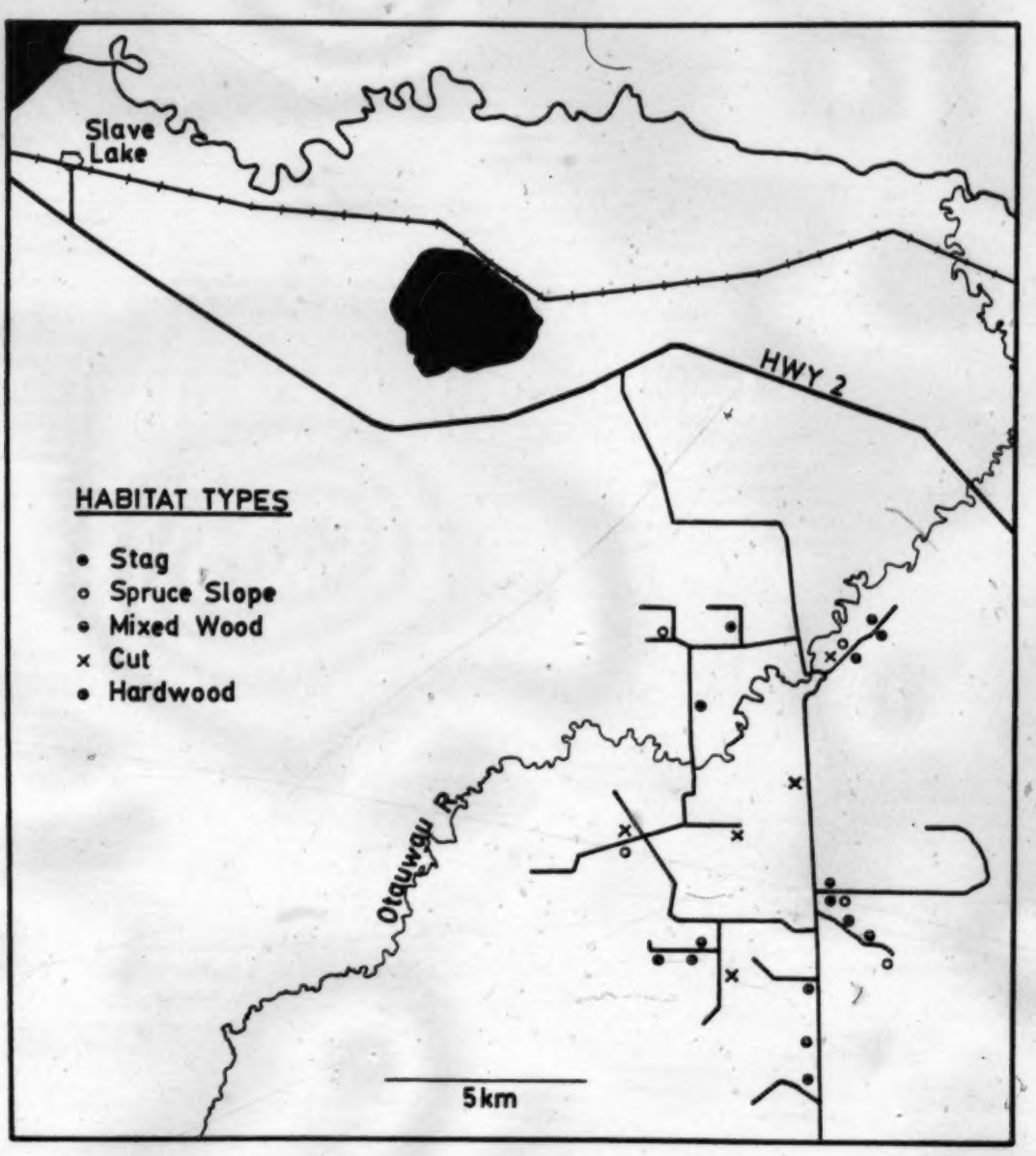
Vegetation Analysis

Five plots, at least 2ha in area, of each habitat type were chosen within an area of approximately 90km² (Fig. 26). Plots with similar density and abundance of tree, shrub and herb species were selected. Snail collections and hare fecal pellet counts were conducted on these 25 sites.

Three methods were used to quantitatively describe the five habitat types. First, trees with a diameter at breast height (DBH) greater than 1cm were censused using the point-centered quarter method (Cottam and Curtis 1956). Twenty points were sampled at regular intervals along a 100m transect established previously for snail and pellet sampling at each of the 25 sites (see below). Data from the five examples of each habitat type were grouped and relative density, relative dominance, relative frequency and an importance value (Cottam and Curtis 1956) were calculated for each tree species within each habitat type. Tree species were ranked by importance value.

The second method, line-intercept (Mueller-Dombois and Ellenberg 1974), was used to describe the shrubs, defined as woody undergrowth less than 1cm DBH and greater than 0.5m tall. Measurements were made with a 50m tape laid over the shrubs between the 25 and 75m marks of the previously-mentioned transects. Data were grouped to produce values of % cover and relative cover for each of the shrub species present on each of the five habitat types. Shrub species were ranked by relative cover.

Figure 26. Location of habitat type plots on the study area.


Slave Lake
HWY 2
HABITAT TYPES
• Stag
o Spruce Slope
• Mixed Wood
× Cut
• Hardwood
Otauwau R.
5km

The third method was an estimate of the extent of cover of herb species within a $1m^2$ quadrat using the Daubenmire cover scale (Mueller-Dombois and Ellenberg 1974). The herb layer was all vegetation less than 0.5m tall. Ten randomly-placed quadrats were examined on each transect, and the data were grouped to produce relative frequency, relative cover, and an importance value (rank) (Wikum and Shanholtzer 1978) for each species in each habitat type.

Vascular plants were identified to species, where practical, with the aid of Moss (1974) and Cormack (1977). Non-vascular plants, and some complex genera of vascular plants were not identified to species. All habitat analyses were performed in mid-August, 1981.

Collection of Terrestrial Gastropods

Gastropods were collected from all 25 habitat plots at intervals from May to October to determine the monthly prevalence and intensity of infection with *P. boughtoni*. On each plot, a 100m transect was marked through the vegetation. Care was taken to ensure that each transect intersected relatively homogeneous habitat along its entire length.

Each month, five random points were chosen along a transect and $0.05m^2$ x 5cm samples of organic litter were cut and removed. In habitats where the litter layer was less than 5 cm, the entire layer was removed down to mineral soil. Gastropods were collected from litter samples by the cold water process (CWP), modified from Beyer and Saari

(1978). Each litter sample was placed in a 0.05m² x 9cm plastic box[6] and thoroughly wetted with pond water. The boxes were covered, and water was added on each of the next two days until the soil was nearly submerged. Gastropods were collected daily for three days from the surface of the litter and sides of the boxes as they climbed to avoid the rising water level.

Three tests of the feasibility and efficiency of this collection technique were performed. First, a comparison was made between the CWP and a dry-sifting technique. Ten paired samples of leaf litter were collected from the same aspen-dominated habitat near Ellerslie, Alberta. One sample of each pair was subjected to the soil-flooding regimen described previously. The other sample was sifted through three screens of decreasing mesh size, and each resulting size class of litter was carefully searched under bright light for gastropods. The results for each group were statistically compared.

The second test was a measure of the efficiency of recovery of the CWP. Ten 0.05m² x 5cm samples of gastropod-free leaf litter were placed in plastic boxes. Five *Discus cronkhitei* and five *Vallonia gracilicosta* were mixed into each box. The soil-flooding procedure was followed and a % recovery of the two snail species determined.

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[6]Wil-hold. Wilson Mfg. Corp., Sunbury, PA.

The ability of terrestrial snails to survive submergence under water for extended periods of time was also tested. Ten individuals of *D. cronkhitei* and *V. gracilicosta* were placed in individual glass vials covered with perforated caps. The vials were filled with aquarium water, then submerged in a dish of water so that none of the snails had access to air. Survival of snails, determined by observation of the heart beating through the transparent shell, was recorded at 24, 48 and 72 hours post-submergence.

All gastropods recovered from transect samples were identified, crushed between glass slides, and examined with transmitted light at 16X magnification for the presence of nematode larvae. Gastropod identifications were made with the aid of Burch (1962) and Pilsbry (1948). Confirmation of gastropod identifications was provided by Mrs. M. F. I. Smith, Mollusc Unit, National Museums of Canada. Representative gastropod specimens have been deposited in the National Museum mollusc collection under accession number 1982-67. The lengths of all *Vertigo gouldi* subspecies were recorded before crushing.

Larvae recovered from gastropods were fixed in hot glycerin-alcohol. Lengths of larvae recovered from naturally-infected *V. gouldi* and experimentally-infected *Vallonia pulchella* were compared as outlined in Chapter I.

Survey of Snowshoe Hares

Infection of *Lepus a. americanus* with *P. boughtoni* in the study area was monitored monthly from January to

December, 1981 by examination of trapped and shot hares of all age groups. Most hares were trapped using unbaited single or double door live traps following the methods of Keith *et al.* (1968). Some hares were collected by shooting, using a .22 rifle. Hares were collected at various sites within the study area where they were abundant, but no effort was made to categorize collected hares by habitat type as their reported home ranges of 2 to 16 ha (Windberg and Keith 1976) would probably encompass more than one habitat type on the study area. Care was taken not to collect hares on or near the habitat plots chosen for gastropod collections. Hares were classified in age groups as adult, litter 1, litter 2, or litter 3, and a sample of each group was collected monthly as it became available through the year. An attempt was made to collect at least 10 individuals of each age group each month. All hares were designated as adults from January until the appearance of the first litter group in late May.

Hares trapped were killed by cervical dislocation. Fresh fecal pellets were collected from beneath the trap, or from the rectum of the hare. All hares were weighed, and the hind foot length of juveniles was measured. Eyes were removed and fixed whole in 10% formalin for subsequent age determination by dried lens weight following Keith *et al.* (1968). Hares were placed into age groups on the basis of body weight, hind foot length and lens weight. The abdominal cavity was opened and gonads were examined to confirm sex of

individuals. The thoracic cavity was opened and the lungs, including half the trachea, were removed. These were rinsed with water, towel-dried, weighed and frozen.

Approximately one gram of fresh feces from each hare was examined by zinc-sulphate flotation (see Chap. I) for *Protostrongylus* larvae. The lungs were thawed and separated into 6 lobes: intermediate (with trachea), left apical, left diaphragmatic, right apical, right cardiac and right diaphragmatic (anatomy after Kozma *et al.* 1974). Each lobe was carefully torn into pieces less than 3mm in diameter under 6.4X magnification. Nematodes were removed, classed as immature (early L5) or adult, counted, sexed and fixed in cold glycerin-alcohol. A random subsample of 10 adult male and ten adult female nematodes from each month was cleared in lactophenol, measured, and compared to Goble and Dougherty's (1943) description of *P. boughtoni*.

The macerated lung tissue from each hare was soaked in 0.85% saline overnight to allow larvae to crawl free of the tissue. The solution was vigorously shaken, poured through a strainer, and the resultant fluid was washed repeatedly by low-speed centrifugation (600 rpm) until most tissue debris had been removed from the plug of larvae at the bottom of the tube. First-stage larvae were counted at 16X magnification and a value of larvae per gram (LPG) of lung tissue was calculated. Also, advanced stages of larvae found in the lung sediment were counted, collected, and prepared (see Chap. I) for identification.

The prenatal young of pregnant snowshoe hares were removed and examined as mentioned in Chapter I to determine whether transplacental transmission of *P. boughtoni* had occurred.

Snowshoe Hare Distribution

The distribution of snowshoe hares within the study area was determined by counting fecal pellets on each of the habitat plot transects, a method adapted from Rogers and Myers (1979). Every month, ten random points were chosen on each of the 25 transects. At each point a $1m^2$ quadrat was placed on the substrate and all snowshoe hare pellets within the quadrat were counted. No attempt was made to exclude old pellets from the count, and only pellets on the surface of leaf litter were included. Counts were taken from May until September, after which leaf fall prevented further enumeration.

Data Analysis

Most statistical analyses were accomplished following the methods outlined in Chapter I. Two-way ANOVA was calculated using a program in BMDP (Dixon and Brown 1979). G-tests were calculated following Sokal and Rohlf (1981).

Results

Vegetation Analysis

The relative density, relative dominance, relative frequency and importance value (I.V.) of all tree species within each habitat type are contained in Appendix IIIa. The magnitude of I.V.'s indicates that in the ST, SS, and HW

habitats, one or two species accounted for most of the total area occupied by trees. In the MW and CUT habitats, there were relatively more species present, with the I.V. shared more evenly among them. In the MW, *Picea glauca* with an I.V. of 99.1 occupied marginally more area than the other species, but in the CUT there was a relatively homogeneous distribution of I.V.

In the ST, SS, MW, and HW habitats, one or two species of shrub provided the majority of cover with the other species occurring less frequently (Appendix IIIb). The fewest species were present in SS (6). In the CUT habitat, the numerous colonizing species produced a relatively balanced cover, with the exception of *Lonicera involucrata*, which accounted for 34.8%.

Appendix IIIc contains the relative frequency, relative cover and importance value for each species of herb in the five habitat types. The species richness of herbs in the ST was low with much cover provided by Bryophyta and lichen. Many more species occurred in the remaining 4 habitats, with much overlap among them. Some short-lived, seasonally-occurring herb species may have been absent in this one-time census.

Assessment of Gastropod Collection Technique

The first test of the CWP collection technique was a comparison with a dry-sifting method. With the latter, a mean of 4.5 live snails was recovered per sample. The CWP recovered a significantly greater mean of 9.1 live snails

($t=-2.42$; $p<0.05$). In addition, the CWP required much less time, a mean of 0.45 hours per sample as opposed to 1.38 hours per sample for the sifting technique. The relative frequencies of snail species recovered were similar with both methods, with the exception of *Vallonia gracilicosta* (Fig. 27). However, one of the sifted samples contained 10 of the 15 individuals found by this technique, and the unusually large recovery resulted in a proportional decrease in the relative frequencies of the other four species.

A test of the efficiency of the CWP indicated that a mean of 94% (range 80-100%) of 50 *D. cronkhitei* and 78% (range 60-100%) of 50 *V. gracilicosta* (5 each in 10 boxes) were recoverable over the three day period.

A test of the ability to tolerate submergence under water for the period required of the CWP demonstrated that 7/10 *D. cronkhitei* and 8/10 *V. gracilicosta* survived. The entire samples of both species survived 24 hours, while 9/10 *D. cronkhitei* and 10/10 *V. gracilicosta* survived 48 hours. Thus, most mortality occurred on the third day of submergence. Snails actively crawled on the vial walls for the first 24 hours, but were less active thereafter.

Survey of Terrestrial Gastropods

A total of 3005 stylommatophoran gastropods was collected in 750 samples from the study area and examined during the 6 month survey period (Table VI). Forty-seven individuals of four species were infected with a *Protostrongylus*-type larva. Of these species, three were of

Figure 27. Relative frequencies of gastropod species recovered from ten paired samples of litter by dry-sifting (a) and the cold water process (b).

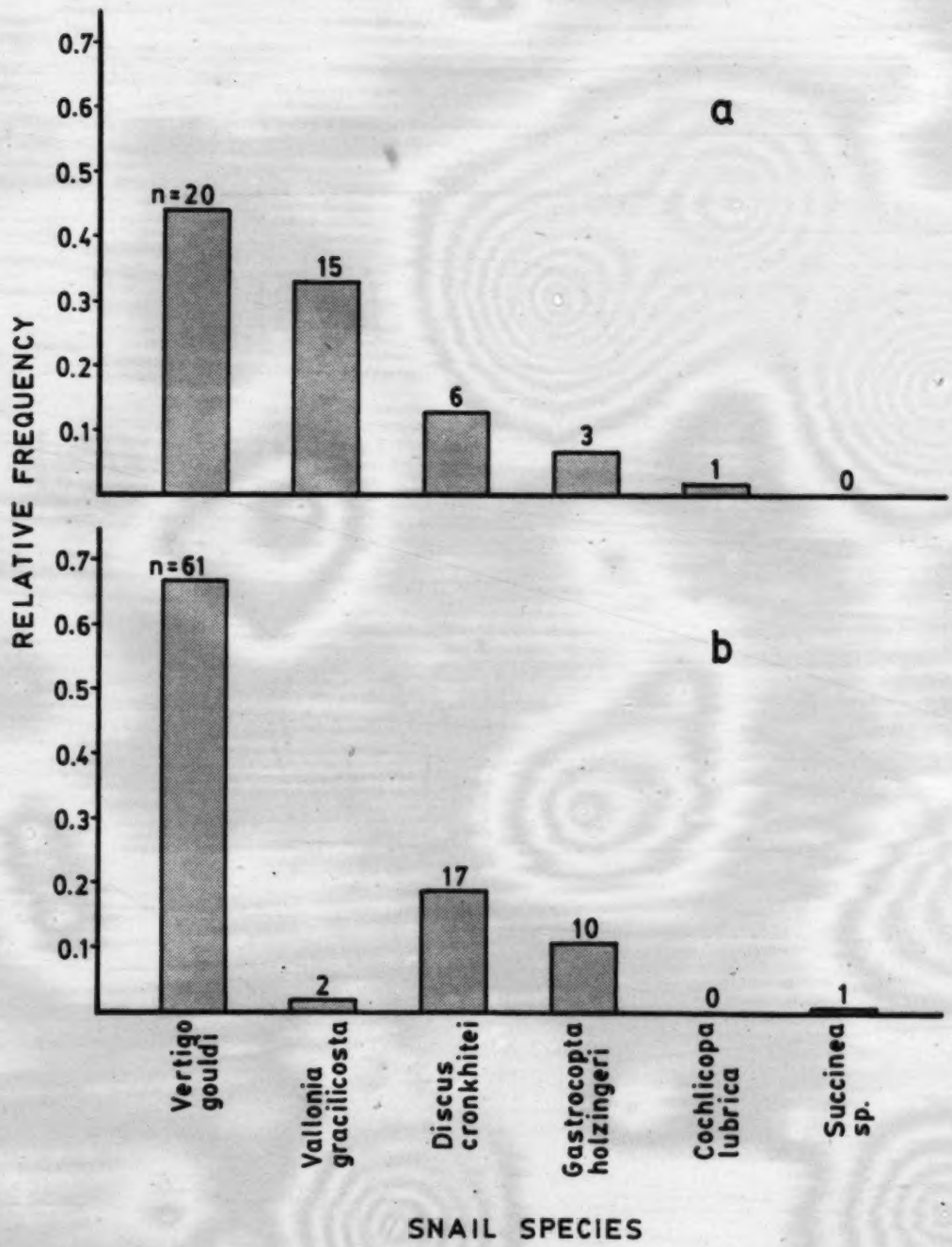

RELATIVE FREQUENCY
0.7
0.6
0.5
0.4
0.3
0.2
0.1
a
n=20
15
6
3
1
0
b
n=61
2
17
10
0
1
Vertigo gouldi
Vallonia gracilicosta
Discus cronkhitei
Gastrocopta holzingeri
Cochlicopa lubrica
Succinea sp.
SNAIL SPECIES

TABLE VI. PREVALENCE OF *PROTOSTRONGYLUS BOUGHTONI* LARVAE IN GASTROPODS COLLECTED FROM THE STUDY AREA IN 1981

SPECIES	NUMBER COLLECTED	NUMBER INFECTED	PREVALENCE (%)	INTENSITY (mean)
Suborder Sigmaurethra				
Family Limacidae				
Deroceras laeve	8	0	0	-
Family Zonitidae				
Euconulus fulvus	356	1	0.3	1
Zonitoides arboreus	9	0	0	-
Vitrina alaskana	34	0	0	-
Retinella electrina	429	0	0	-
Family Endodontidae				
Discus cronkhitei	1005	0	0	-
Punctum minutissimum	149	0	0	-
Suborder Orthourethra				
Family Pupillidae				
Vertigo gouldi subspp.	905	44	4.9	1.6
Vertigo ovata	22	1	4.6	1
Columella edentula	52	1	1.9	1
Family Valloniidae				
Zoögenetes harpa	2	0	0	-
Family Cionellidae				
Cochlicopa lubrica	33	0	0	-
Suborder Heterurethra				
Family Succineidae				
Succinea sp.	1	0	0	-
TOTAL	3005	47	1.6	1.1

the family Pupillidae and the fourth belonged to the family Zonitidae. Larvae recovered from these snails were examined, and lengths were statistically compared to lengths of *P. boughtoni* L3's. No morphological or length differences between the two groups were determined (see Chap. I), and hereafter all larvae collected from naturally-infected snails will be referred to as *P. boughtoni*.

The method of examining snails by crushing was not effective in recovering first- and early second-stage larvae as these were transparent in the foot tissue. Few late second-stage larvae were found (5), so in further analyses all larvae recovered from snails were regarded as infective.

Percent prevalence of *P. boughtoni* in susceptible snails varied from 0.3 to 4.9. However, in each of the cases of *Euconulus fulvus*, *Vertigo ovata* and *Columella edentula*, only one individual of the total sample was infected. Based on these data, it appeared that of the actual intermediate hosts of *P. boughtoni*, only *Vertigo gouldi* subspecies were infected in sufficient abundance to play a significant role in transmission of the parasite. Subsequent analyses in this section will be restricted to *V. gouldi*; the other intermediate host species will be considered further in the next section (CONCLUSIONS).

V. gouldi was found in each of the habitat types (Table VII). The smallest individuals of *V. gouldi* and *C. edentula* (<750um) were indistinguishable from each other on the basis of form or color. To simplify further analysis, all such

TABLE VII. PREVALENCE AND INTENSITY OF INFECTION OF *PROTOSTRONGYLUS BOUGHTONI* IN *VERTIGO GOULDI* FROM EACH HABITAT TYPE

HABITAT	NUMBER COLLECTED	NUMBER INFECTED	PREVALENCE (%)	MEAN INTENSITY (RANGE)
STAG	45	2	4.4	1(1)
SPRUCE SLOPE	389	21	5.4	1.7(1-11)
MIXED WOOD	217	11	5.1	1.5(1-4)
CUT	181	7	3.9	1.1(1-2)
HARD-WOOD	73	3	4.1	2.7(1-6)

individuals were classified as *V. gouldi* in recognition of that species greater abundance. Any resultant error will likely be minimal. A G-test of independence indicated that there was no significant difference in prevalence of infection with *P. boughtoni* due to habitat type ($G=0.78$; $p>0.05$). Similarly, a Kruskal-Wallis test indicated that there was no significant difference in intensity of *P. boughtoni* infection due to habitat type ($H=0.75$; $p>0.05$; median intensity in all habitat types = 1)

The potential for transmission of *P. boughtoni* in the different habitat types was likely a function of the abundance of *V. gouldi* in those areas since prevalence and intensity were constant. The abundance of *V. gouldi* varied significantly between habitat types ($H=110.14$; $p<0.001$). The snails appeared to have an aggregated distribution on each of the habitat types (variance > mean) which did not approximate a negative binomial distribution (G-tests; $p<0.05$). The actual distributions were not determined as there appeared to be no positive association between size of snail aggregations and mean prevalence of infection with *P. boughtoni* ($G=12.91$; $p>0.05$)(Fig. 28).

The arithmetic mean densities of *V. gouldi*, as well as an index of dispersion for the distributions on each habitat type, are presented in Table VIII. The parameter "b" of Taylor's power law was chosen as a measure of the degree of clumping (Elliott 1977). A value for b of 0 indicates a regular distribution, 1 indicates a random distribution and

Figure 28. Prevalence of *Protostrongylus boughtoni* infection in *Vertigo gouldi* aggregations.

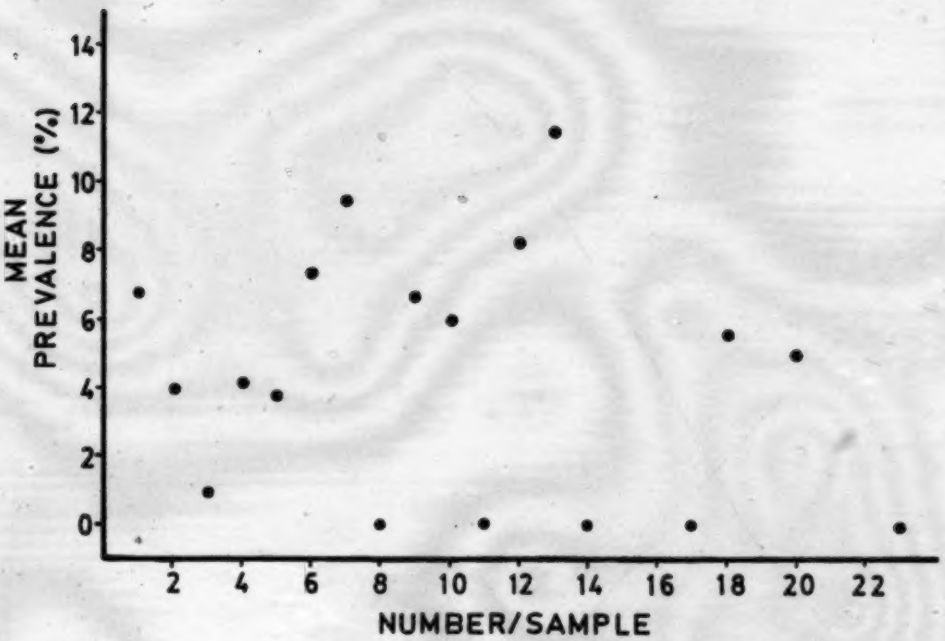

MEAN
PREVALENCE (%)
14
12
10
8
6
4
2
0
2
4
6
8
10
12
14
16
18
20
22
NUMBER/SAMPLE

TABLE VIII. MEAN DENSITY AND DISPERSION OF *VERTIGO GOULDI* ON EACH HABITAT TYPE. DISPERSION IS INDICATED BY THE PARAMETER b OF TAYLOR'S POWER LAW

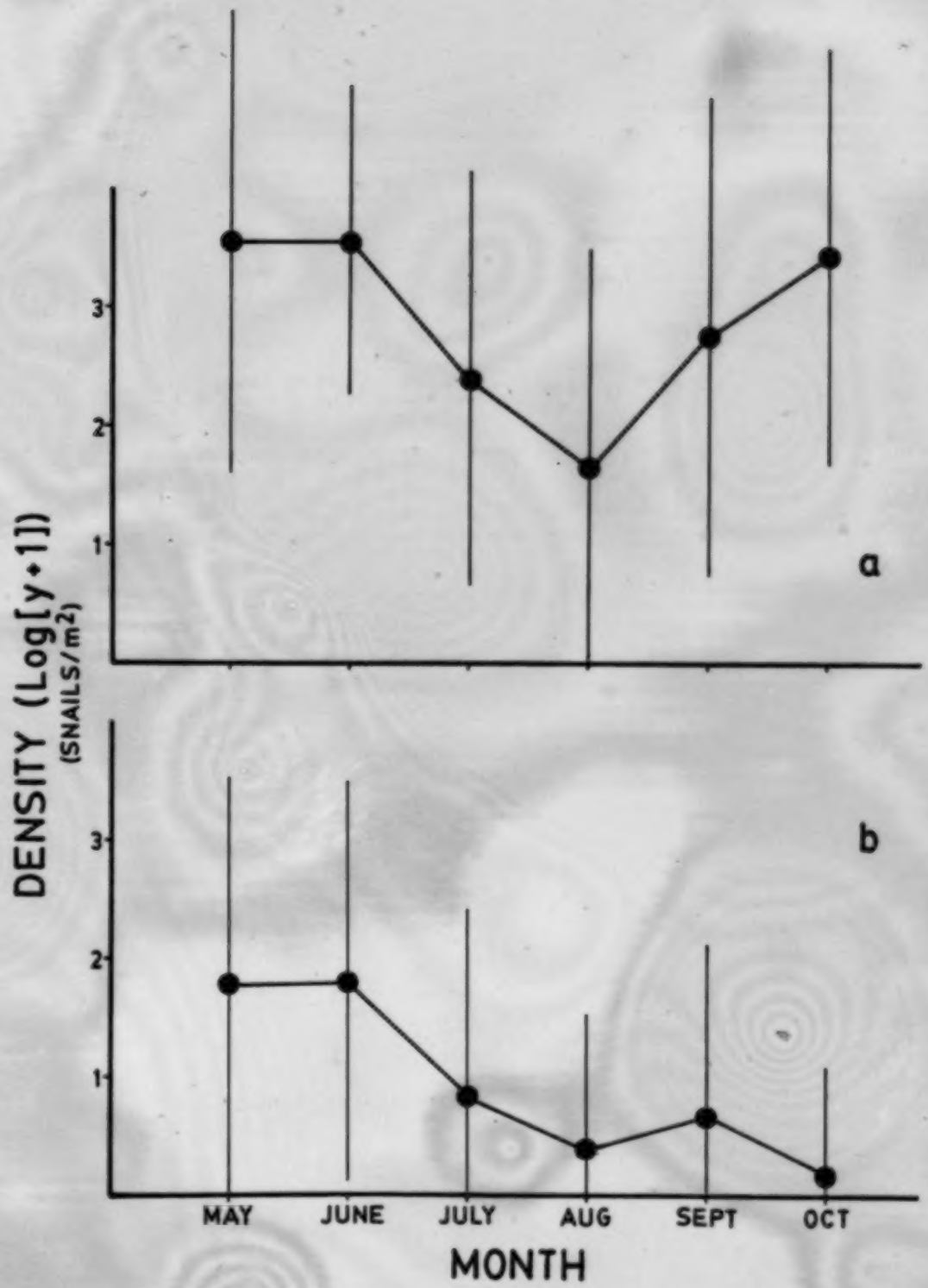
HABITAT TYPE	MEAN DENSITY (SNAILS/m^2)	DISPERSION (b)
STAG	5.87	1.39
SPRUCE SLOPE	53.33	1.83
MIXED WOOD	28.81	2.14
CUT	24.93	1.69
HARD-WOOD	9.60	1.38

values above 1 indicate an aggregated distribution. On each habitat type, b was greater than 1. It is acknowledged that the arithmetic mean densities of snails may not accurately reflect the actual densities on each habitat type given the possibility of differences in snail dispersion and influences of seasonal variation in abundance. However, since the relative magnitudes of snail density on each habitat type follow the same order as total numbers collected, these means may be of value in lieu of more accurate descriptive statistics.

Neither overall prevalence or intensity of infection of *V. gouldi* with *P. boughtoni* fluctuated monthly from May until October ($G=2.48$; $p>0.05$, and, $H=2.10$; $p>0.05$). Monthly fluctuations in *V. gouldi* abundance were detected only on the SS and HW habitats ($H=22.89$; $p<0.001$, and, $H=15.72$; $p<0.01$). On both habitats, snail density declined from June to August (Fig. 29). Density increased through September and October on SS, but remained depressed on HW.

An attempt was made to examine the relationship between age classes of *V. gouldi* and prevalence of infection with *P. boughtoni*, assuming that snail shell length was related to snail age. All *V. gouldi* collected were assigned to five arbitrary size classes with 500um intervals, after Platt (1978). Snails of the smallest size class (<500um) were uninfected, and there was no significant difference in prevalence of infection among the remaining 4 classes ($G=1.18$; $p>0.05$). Neither was there a significant difference

Figure 29. Mean monthly population densities of Vertigo gouldi in Spruce Slope (a) and Hardwood (b) habitats from May to October, 1981. Bars above and below points represent one standard deviation.


DENSITY (Log[y+1])
(SNAILS/m²)
3
2
1
a
b
MAY
JUNE
JULY
AUG
SEPT
OCT
MONTH

in intensity ($H=1.26$, $p>0.05$).

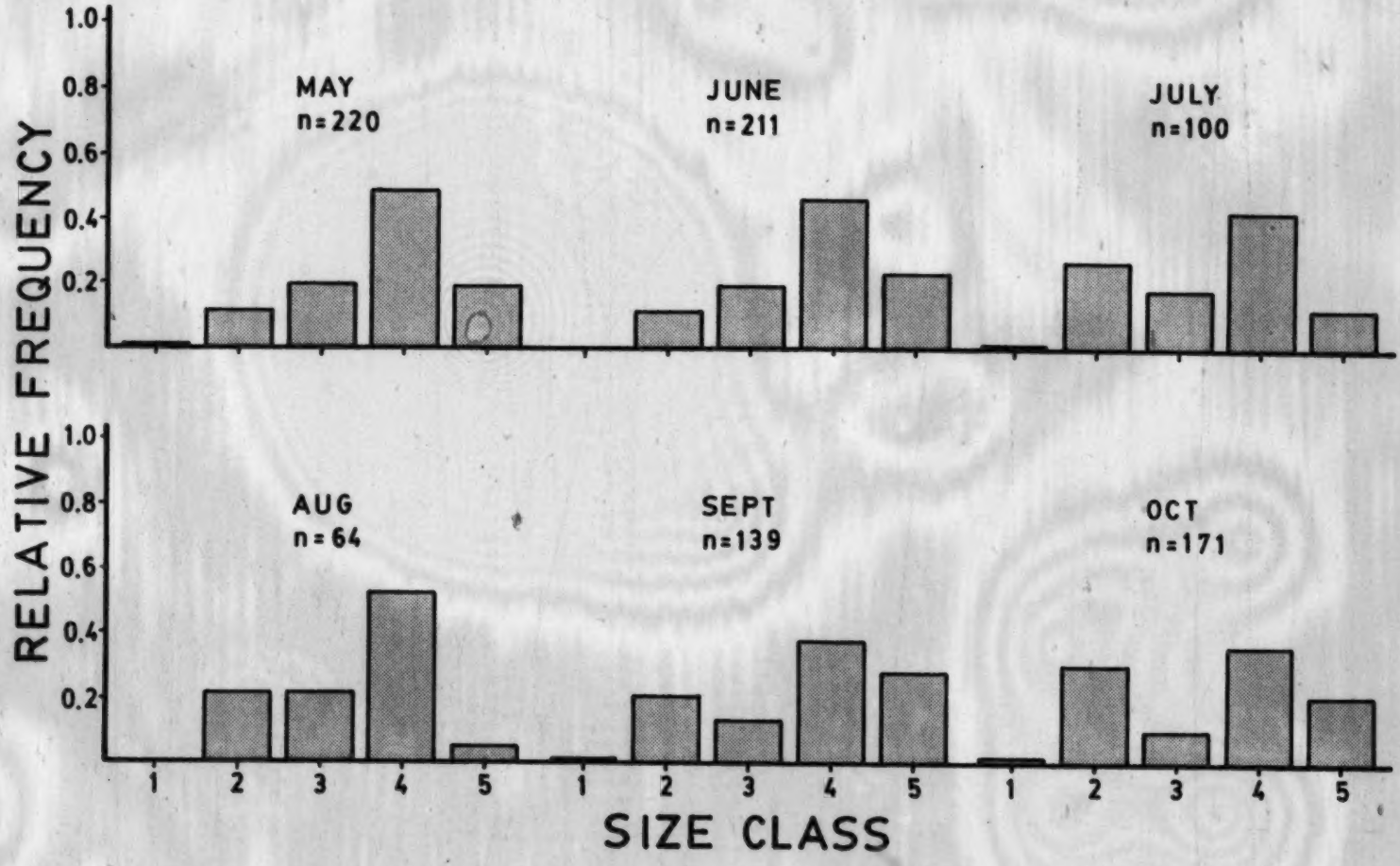
The distributions by size class of all *V. gouldi* collected each month are presented in Figure 30. The distributions were unimodal until July, at which time they became more or less bimodal, with the exception of August. This may indicate that young *V. gouldi* begin recruitment into the population between June and July and continue until October, since the first mode remained at size class 2. There appeared to be no die-off of older (=larger) individuals. The difference in August's distribution may have been due to effects of the decreased sample size for that month.

Survey of Snowshoe Hares

A total of 261 hares from 12 months in 1981 was examined for *P. boughtoni*. Overall prevalence of infection was 92.3%, and the mean intensity was 27.3 with a range of 1 to 284. Sample sizes from each month for each age group are presented in Appendix IV.

Prevalence of post-larval *P. boughtoni* in adult snowshoe hares from the study area was 100% in every month. All young of the year hares greater than approximately 60 days of age were infected, and the youngest hare with *P. boughtoni* was approximately 23 days old. The youngest hare with a patent lungworm infection was approximately 40 days old. The prevalence of *P. boughtoni* in juvenile hares from August to December was 100%.

Figure 30. Monthly relative frequencies of Vertigo gouldi size classes from May to October, 1981. Size classes: 1 = less than 500um; 2 = 500-999um; 3 = 1000-1499um; 4 = 1500-1999um; 5 = 2000um and greater.


RELATIVE FREQUENCY
1.0
0.8
0.6
0.4
0.2
MAY
n=220
JUNE
n=211
JULY
n=100
AUG
n=64
SEPT
n=139
OCT
n=171
1 2 3 4 5 1 2 3 4 5 1 2 3 4 5
SIZE CLASS

To determine whether intensity of infection with *P. boughtoni* differed between male and female hares, t-tests were performed within adult and grouped juvenile samples for each month. These indicated that there was no significant difference in intensity between the sexes for either age group in any month ($p>0.05$). To determine whether the sex ratio of *P. boughtoni* adults differed between male and female hares, t-tests were performed on the intensity of female nematodes between male and female hares of adult and grouped juvenile samples for each month. There was no significant difference in female *P. boughtoni* intensity between male and female hares from any group or time period ($p>0.05$). Therefore, since intensity did not differ between male and female hares, it follows that the sex ratio did not either. These initial tests established that there were no differences in intensity or sex ratio of nematodes between hare sexes and, therefore, data from male and female hares were grouped in subsequent analyses.

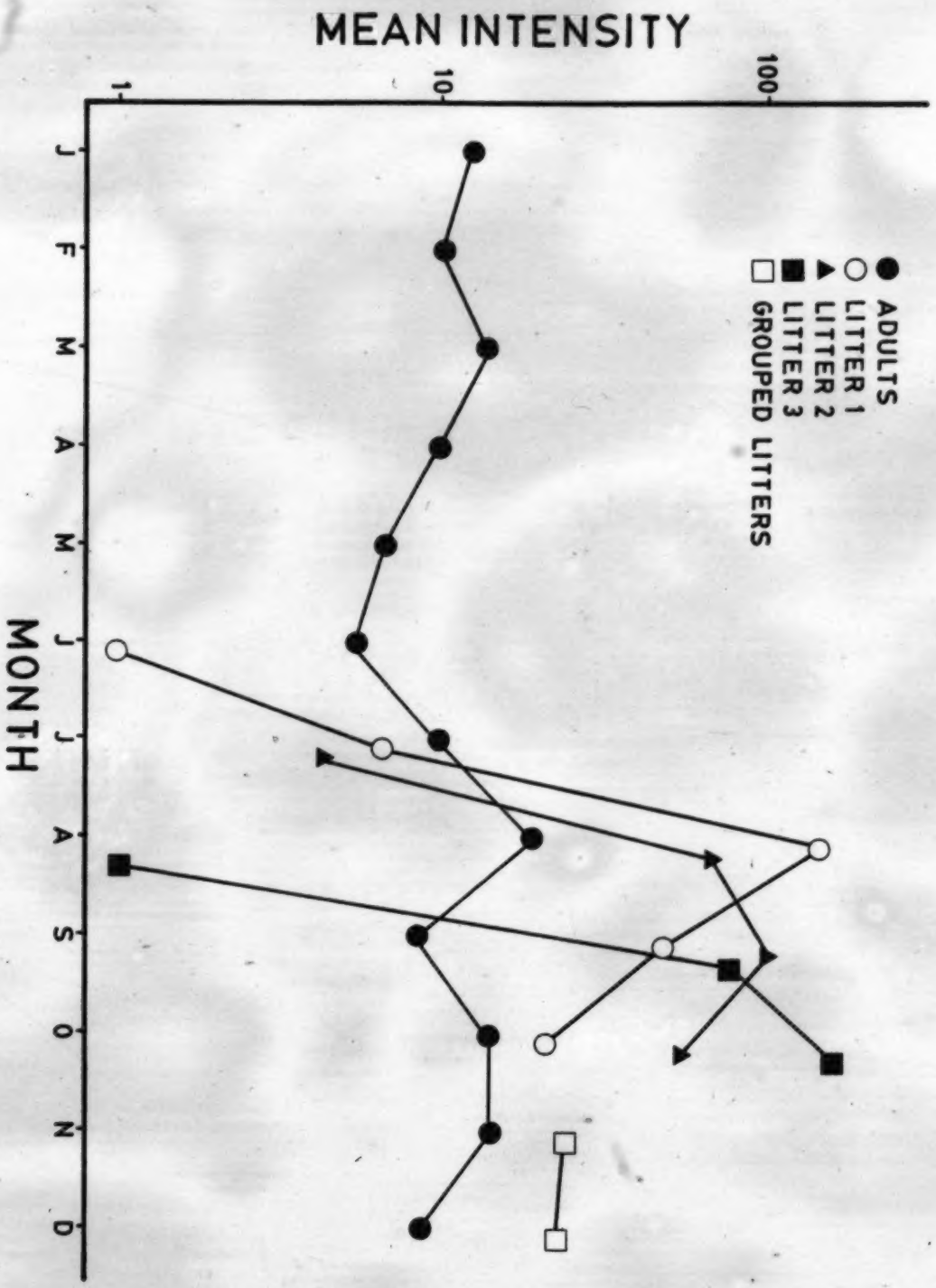
Juvenile hares were separable into three litter groups on the basis of ageing techniques until November, at which time these techniques, based on relative size or weight, could not differentiate among fully-grown juveniles. Prevalence of *P. boughtoni* infection in the first litter was 0% in May (0/2), 30% in June (3/10) and 70% in July (7/10). Prevalence in the second litter was 0% in June (0/2) and 50% in July (6/12). The third litter group was very small and did not appear until August, at which time all young of the

year were infected, as mentioned previously.

Intensity of *P. boughtoni* in adults varied monthly ($H=29.21$; $p<0.01$)(Fig. 31), declining from March until June, then increasing to a maximum in August. Mean intensity in adult hares never exceeded 20; maximum intensity was 41 from an August hare.

Intensity varied significantly by month within the first two litter groups ($H=24.70$; $p<0.001$, and, $H=16.64$; $p<0.001$) but not within the third litter group ($H=4.30$; $p>0.05$)(Fig. 31). Lack of significance in this test was likely due to extremely small sample sizes (1 in August, 2 in September, 3 in October), but since the trend exhibited by the data was consistent with the first two litters, they were included in the figure for comparison. Mean intensity in each litter group increased exponentially from the first month that members became infected, to a peak of over 100 two months later. The peak mean intensities for the first two litter groups were separated by one month; this was the approximate period between parturition of the litters for adult females. The third litter group likely reached peak mean intensity in October because transmission of the parasite in winter months was assumedly curtailed. Data from all November and December juveniles were combined, and they indicated a continued reduction in mean intensity over those months (Fig. 31). Maximum intensity was 284 for a third-litter juvenile in October.

Figure 31. Monthly mean intensity of Protostrongylus boughtoni infection in each snowshoe hare age group in 1981. Standard deviations for means are listed in Appendix V.


MEAN INTENSITY
1
10
100
MONTH
J
F
M
A
M
J
J
A
S
O
N
D
ADULTS
LITTER 1
LITTER 2
LITTER 3
GROUPED LITTERS

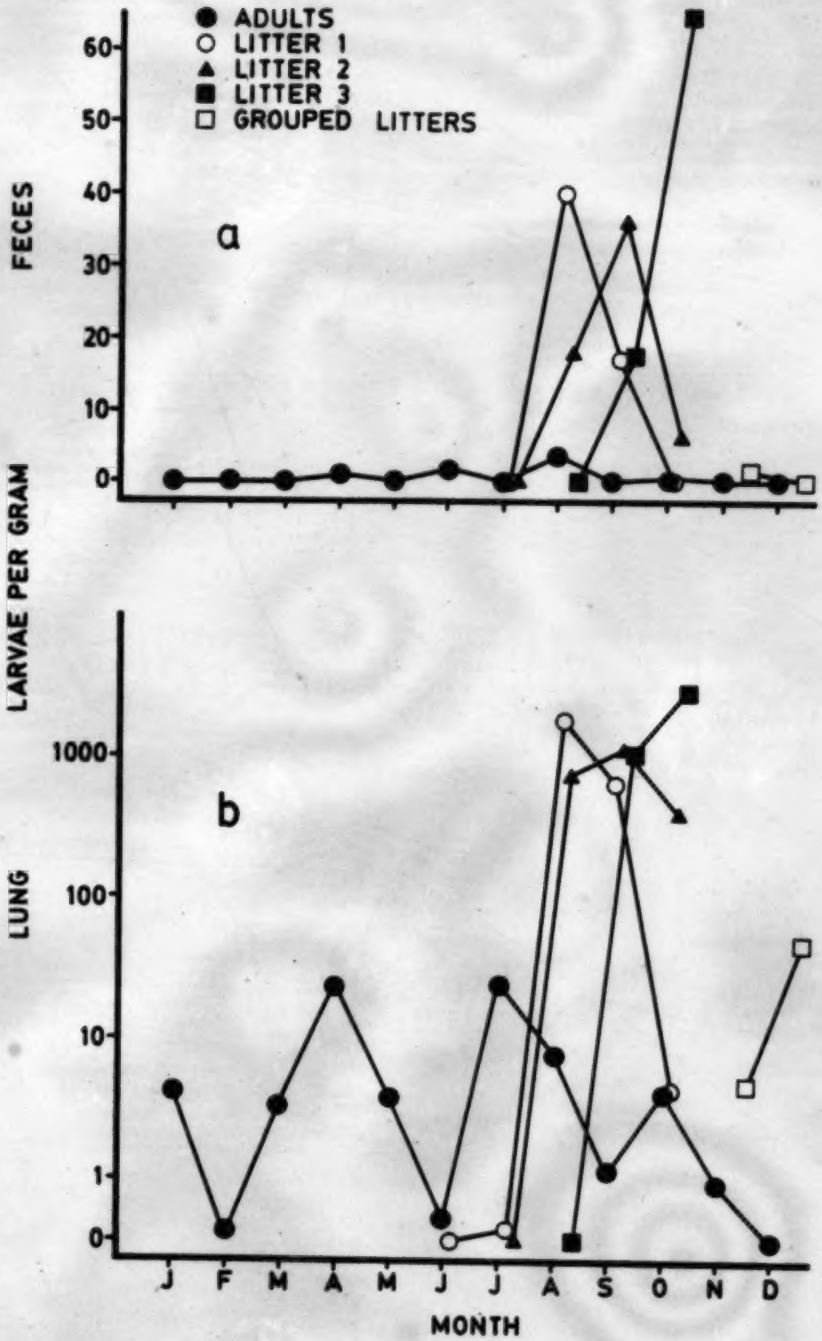
Numbers of first-stage larvae in the feces of each age group of hares are presented in Figure 32a. Median values are plotted as the extremely high variance in these data disallowed the use of means as descriptive statistics.

There was significant monthly variation in L1 output among adult hares ($H=26.76$; $p<0.01$). Median LPG of feces, which was very low in every month, peaked in August coincidental with maximum mean intensity of *P. boughtoni* infection (Fig 31). Maximum larval output for an adult hare was 51 LPG of feces recorded in April.

First and second litter larval output also varied with time ($H=17.01$; $p<0.01$, and, $H=15.32$; $p<0.01$) and peaked coincidentally with mean intensity of *P. boughtoni*. Larval output of the third litter did not show significant change, but the pattern was similar to that of the first two litters. Larval output of grouped juveniles in November and December was relatively low. Maximum LPG of feces from a juvenile was 416 recorded from a first litter hare in August.

There was monthly variation in median LPG of lung tissue in adult hares ($H=27.11$; $p<0.01$)(Fig. 32b), but it did not reflect the pattern of mean intensity of *P. boughtoni* infection (Fig. 31). This reflection, however, was seen in the data for the first two litters ($H=22.19$; $p<0.001$, and $H=16.43$; $p<0.001$)(Fig. 32b). There was no significant monthly variation in this parameter in the third litter group or between grouped juveniles of November and

Figure 32. Monthly median numbers of first-stage larvae per gram of feces (a) and lung tissue (b) from each snowshoe hare age group in 1981.


ADULTS
LITTER 1
LITTER 2
LITTER 3
GROUPED LITTERS
FECES
LARVAE PER GRAM
a
b
LUNG
60
50
40
30
20
10
0
1000
100
10
1
J F M A M J J A S O N D
MONTH

December ($p>0.05$). Maximum LPG of lung tissue for an adult hare was 504 recorded in August, and for a juvenile was 3486 recorded from a third litter hare in October.

Linear regressions were calculated for intensity of adult *P. boughtoni* in the lungs on LPG of feces for adults and grouped juvenile hares (Fig. 33) to determine if fecal output of L1's could be an accurate predictor of the intensity of infection. In adult hares, only 5.7% of the variability in intensity could be accounted for by this regression ($t=2.92$; $p<0.01$). In juvenile hares, however, 57.6% of the variability in intensity could be accounted for by this relation ($t=11.66$; $p<0.001$). The slopes of the regressions for adult and juvenile hares were not homogeneous ($F=20.08$; $p<0.001$).

Linear regressions were also calculated for intensity of adult *P. boughtoni* in the lungs on LPG of lung tissue for adults and grouped juvenile hares (Fig. 34) to determine if number of tissue L1's was a more accurate predictor of the intensity of infection. In adult hares, 28.3% of the variability in intensity could be accounted for by this regression ($t=7.47$; $p<0.001$). In juvenile hares, 70.9% of the variability in intensity could be accounted for by this relation ($t=15.61$; $p<0.001$). The slopes of the regressions for adult and juvenile hares were not homogeneous ($F=20.55$; $p<0.001$).

Fourth-stage larvae of *P. boughtoni* were found in the lungs of adult hares from every month but May (Fig. 35).

Figure 33. Number of adult Protostrongylus boughtoni in the lungs of adult (a) and juvenile (b) snowshoe hares versus number of first-stage larvae per gram of feces, with regression equations.

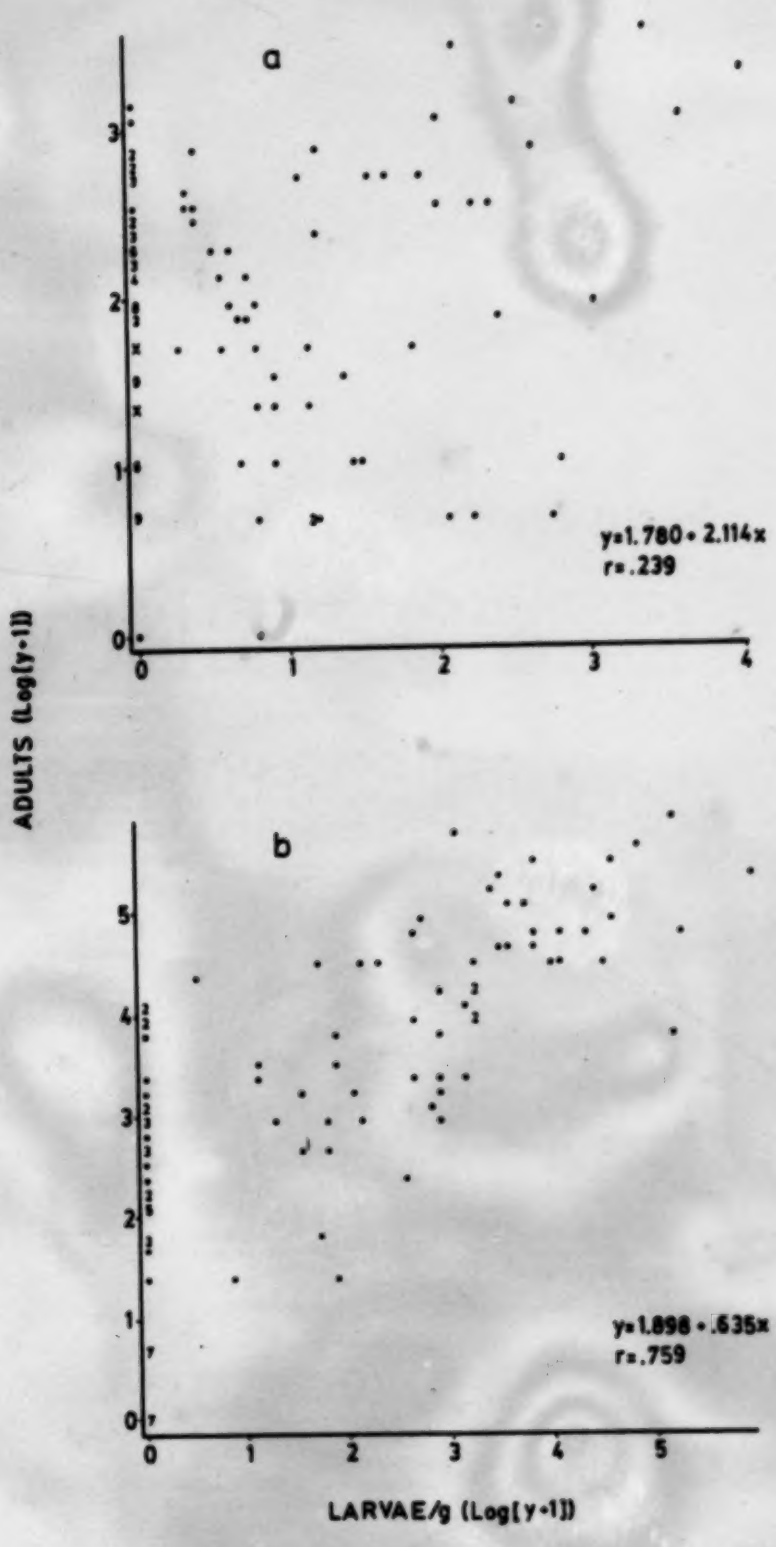

a
y=1.780 + 2.114x
r=.239
b
y=1.898 + .635x
r=.759
ADULTS (Log[y+1])
LARVAE/g (Log[y+1])

Figure 34. Number of adult Protostrongylus boughtoni in the lungs of adult (a) and juvenile (b) snowshoe hares versus number of first-stage larvae per gram of lung tissue, with regression equations.

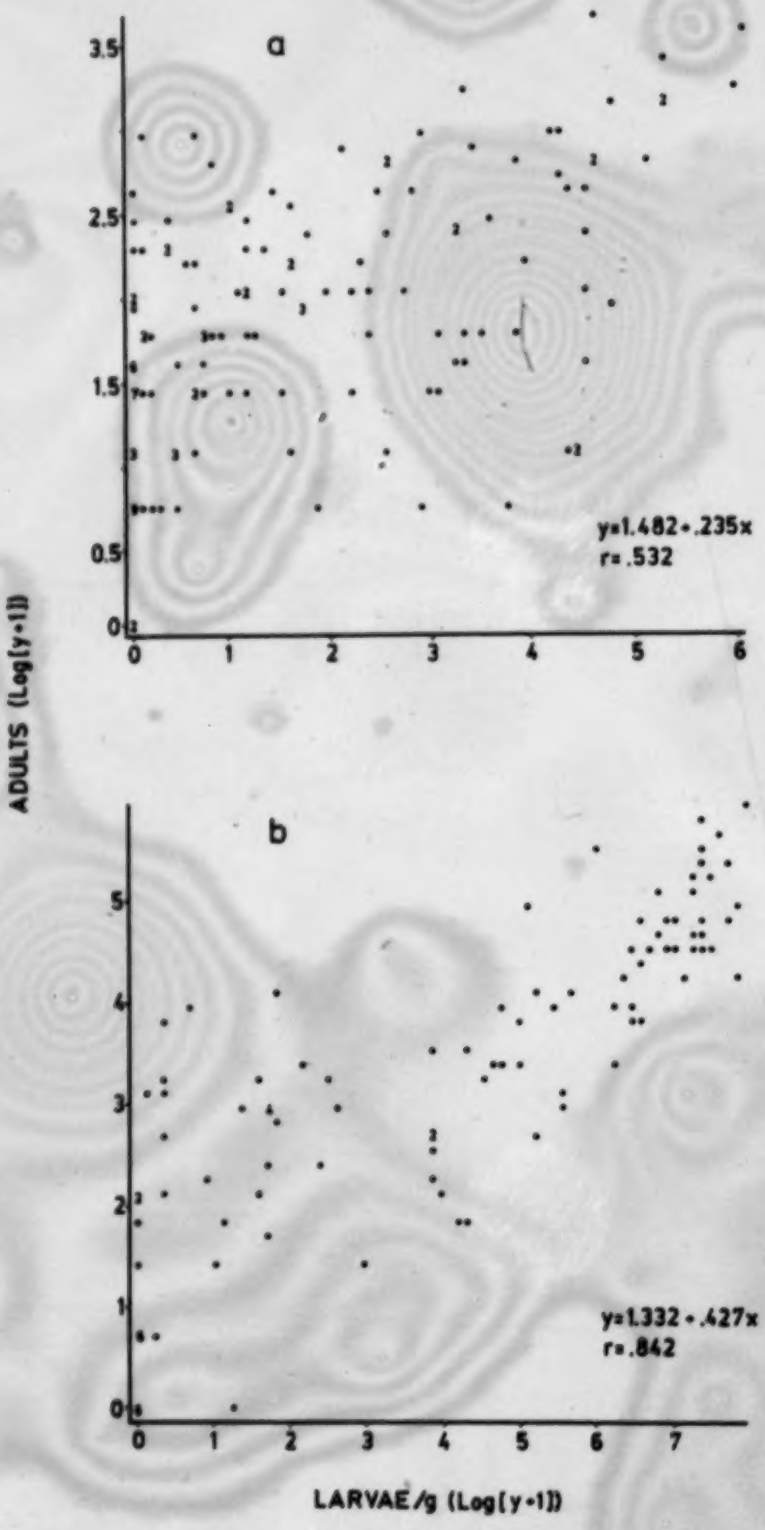
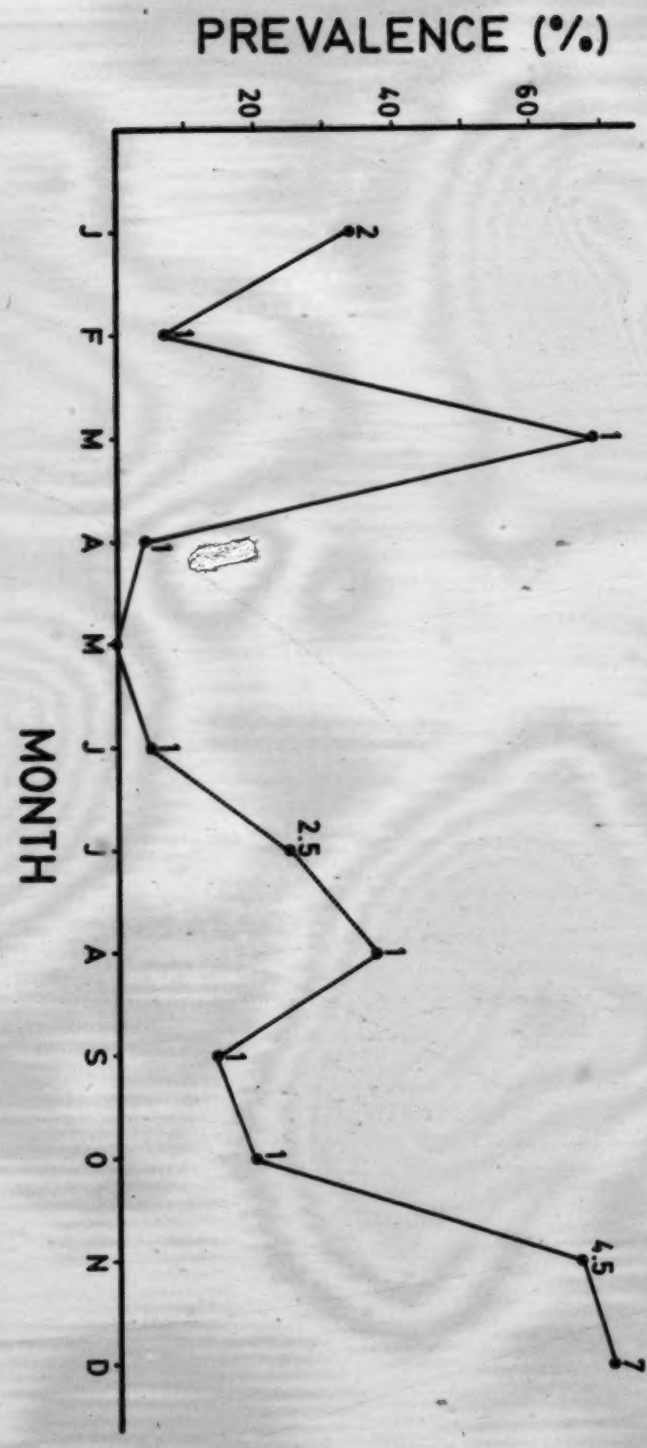

a
y=1.482+.235x
r=.532
b
y=1.332+.427x
r=.842
ADULTS (Log[y+1])
LARVAE/g (Log[y+1])

Figure 35. Monthly prevalence of fourth-stage Protostrongylus boughtoni larvae in the lungs of adult snowshoe hares in 1981. Median intensities are presented above data points.


PREVALENCE (%)
20
40
60
MONTH
J
F
M
A
M
J
J
A
S
O
N
D
2
1
1
1
2.5
1
1
1
4.5
7

Percent prevalence varied monthly up to 69% (G=22.61; $p<0.05$) with peaks in March and December. Intensity was commonly low but also varied monthly (H=32.27; $p<0.001$), and was consistently highest over the period of November to January.

Neither prevalence nor intensity of infection of juvenile hares with L4's varied by month or between litter groups or between litter groups and November-December grouped juveniles (G- and Kruskal-Wallis tests; $p>0.05$). Thus, data for all juveniles were combined. The grand prevalence was 27.5%, while the median intensity was 3. L4's were recovered from juvenile hares trapped in winter months, even though the ground was frozen since early November and there had been a heavy cover of snow since late in that month. Both factors would assumedly prevent transmission of the parasite from snails to hares.

There was no significant difference in the distribution of *P. boughtoni* among the lung lobes between adult and juvenile hares (G-tests for each lobe; $p>0.05$). Thus, all data were grouped (Table IX). Of 6673 immature and adult *P. boughtoni* recovered from 241 infected hares during the study, 71% were found in the diaphragmatic lobes. There was no significant preference for either right or left diaphragmatic lobe, and there was no significant preference for either right or left apical lobe (G=1.52; $p>0.05$, and, G=1.22; $p>0.05$). Immature *P. boughtoni* (early L5's) were most often found coiled in the parenchyma near the surface

TABLE IX. DISTRIBUTION OF ADULT AND IMMATURE *PROTOSTRONGYLUS BOUGHTONI* IN THE LOBES OF NATURALLY-INFECTED *LEPUS AMERICANUS* LUNGS

LOBE	TOTAL WORMS RECOVERED	% OF GRAND TOTAL
INTERMEDIATE	618	9.26
LEFT APICAL	436	6.53
LEFT DIAPHRAGMATIC	2337	35.02
RIGHT APICAL	468	7.01
RIGHT CARDIAC	409	6.13
RIGHT DIAPHRAGMATIC	2405	36.04
TOTAL	6673	

of lobes.

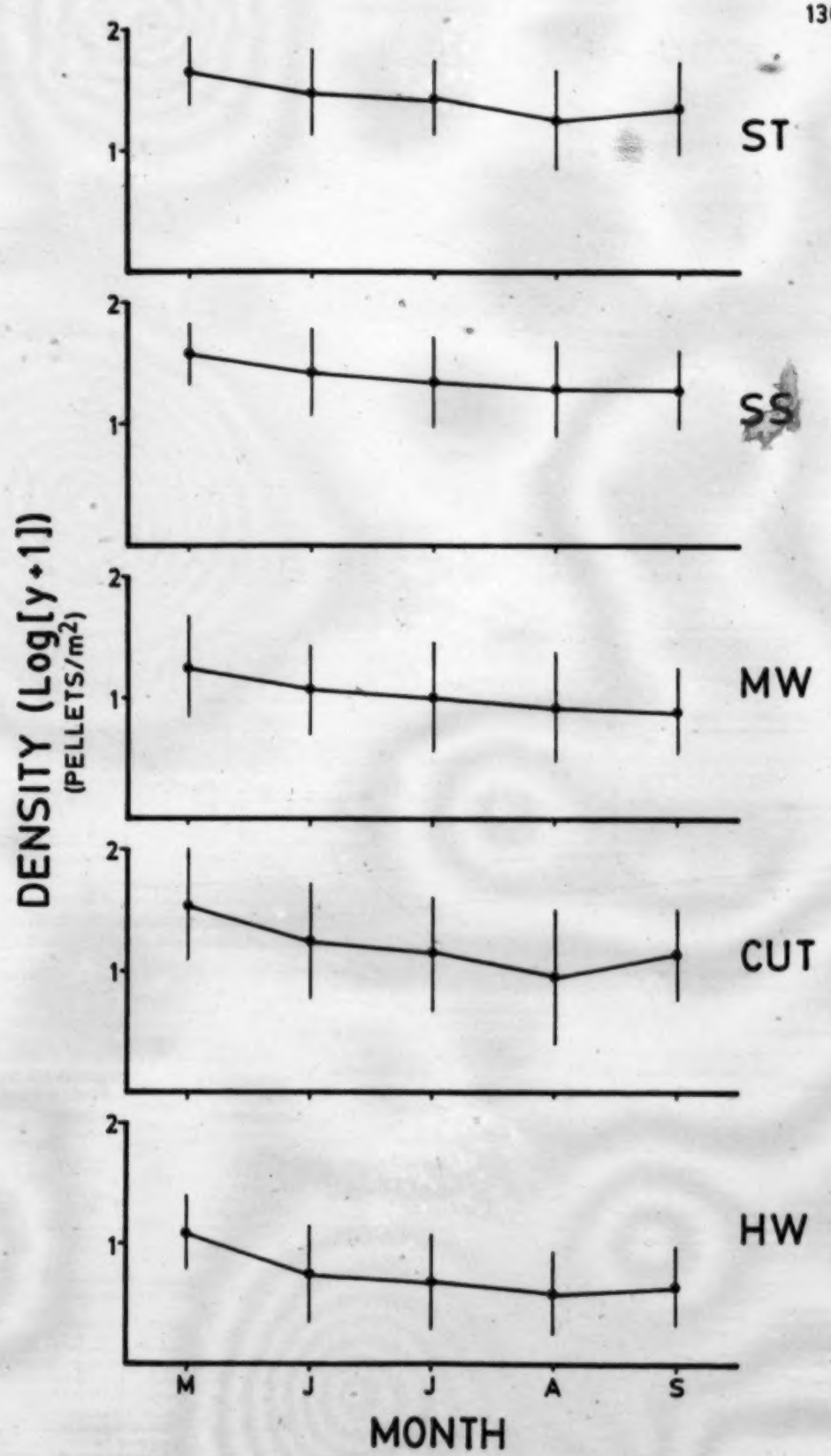
The sex ratio of adult *P. boughtoni* in the lungs of adult and juvenile hares was examined. There was no significant monthly variation in sex ratio of adult *P. boughtoni* within adult hares ($G=19.25$; $p>0.05$). Similarly, there was no significant sex ratio variation in juveniles grouped by month of age ($G=0.60$; $p>0.05$). A G-test on data from all adult hares versus all juvenile hares revealed no significant difference in the sex ratios of worms between these two groups ($G=2.60$; $p>0.05$). Therefore, data from all infected adults and juveniles were combined and a t-test revealed no significant difference between mean numbers of male and female *P. boughtoni* ($t=1.25$; $p>0.05$).

Digestions of 44 prenatal young from pregnant hares trapped from April to June, and 4 stillborn young from a hare trapped in June, resulted in no recovery of *Protostrongylus* larvae (Appendix II).

Snowshoe Hare Distribution

Snowshoe hare fecal pellets in a total of 1250 quadrats from the 25 transects were counted. Two-way analysis of variance on log-transformed pellet counts indicated that both month ($F=40.50$) and habitat type ($F=123.99$) were significant ($p<0.001$) factors in the density of pellets observed (Fig. 36). Densities declined in all habitat types from May until August. There were slight increases in pellet densities in the ST, CUT and HW habitats in September which did not occur in SS and MW.

Figure 36. Monthly mean snowshoe hare fecal pellet densities on each habitat type from May to September, 1981. Bars above and below points represent one standard deviation.


2
1
ST
SS
MW
CUT
HW
DENSITY (Log[y+1])
(PELLETS/m²)
M
J
J
A
S
MONTH

The mean pellet counts for each habitat type in a month were divided by the sum of all habitat means for that month and multiplied by 100 to produce a % of total pellets found per habitat type for ranking purposes (Table X). The percentage of pellets was consistently lowest in the HW habitat, where it was always less than 8. Next in order of increasing pellet density was MW, where the percentage was repeatedly less than 14. CUT was the next highest habitat type in all months except May, when the percentage of total pellets was highest of all habitats at 29.87. SS had a consistently lower percentage than ST, ranging from 23.02 to 31.01, except in August when ST was slightly lower. ST had the highest percentage in three of the five months. If % of total pellets found per habitat type is a reliable indicator of relative use by snowshoe hares, then relative habitat use from spring to early fall was of the following decreasing order: ST,SS,CUT,MW,HW.

Discussion

Estimates of the distribution and abundance of terrestrial gastropods are difficult to make and the methods employed in past studies often contained elements of bias of unknown nature (Bishop 1977; Boag 1982). Some objectives of the present study were to measure the monthly abundance of intermediate hosts of *P. boughtoni* in different habitats, and to determine the extent to which they were infected with the parasite. Thus, a reliable quantitative approach was essential to meaningful comparisons of data both spatially

TABLE X. MEAN MONTHLY NUMBERS OF SNOWSHOE HARE FECAL PELLETS/m^2 IN EACH HABITAT TYPE. % OF TOTAL PELLETS IS THE HABITAT TYPE MEAN DIVIDED BY THE MONTHLY SUM OF MEANS AND MULTIPLIED BY 100

HABITAT TYPE	MONTH									
	MAY		JUNE		JULY		AUGUST		SEPTEMBER	
	MEAN	% OF TOTAL PELLETS	MEAN	% OF TOTAL PELLETS	MEAN	% OF TOTAL PELLETS	MEAN	% OF TOTAL PELLETS	MEAN	% OF TOTAL PELLETS
STAG	53.00	26.83	39.72	30.89	34.82	31.54	27.16	29.96	32.40	33.71
SPRUCE SLOPE	45.48	23.02	37.48	29.15	31.78	28.79	27.88	30.76	29.80	31.01
MIXED WOOD	24.66	12.48	16.38	12.74	14.86	13.46	12.04	13.28	9.60	9.99
CUT	59.26	29.87	27.50	21.39	22.34	20.24	19.36	21.36	19.26	20.04
HARD-WOOD	15.14	7.59	7.50	5.83	6.60	5.98	4.20	4.63	5.04	5.24
SUM	197.54		128.58		110.40		90.62		96.10	

and temporally. The method chosen, I believe, was the most appropriate given these requirements.

Soil sampling is an accurate method of estimating total numbers, species composition and age distribution of terrestrial gastropods (South 1964; Hunter 1968; Mason 1970; Newell 1971; Bishop 1977; Beyer and Saari 1978). Random quadrat sampling provides information on spatial dispersion, allows a level of precision on the population estimate and allows inter-site comparisons (Bishop 1977). The efficiency of soil sampling is easily tested by introducing a known number of gastropods into a "clean" sample, followed by the recovery technique (South 1964; Hunter 1968: Mason 1970; present study). However, Bishop (1977) indicated that quadrat sampling may underestimate species with strong micro-habitat preferences such as slugs, a statement reiterated by Boag (1982) who, in addition, proposed possible biases based on differential distribution of life stages, differential visibility among species due to size or fragility of shell, and differential decay if only shells are counted.

Four major methods are commonly used to separate gastropods from soil samples: dry-sifting (Bishop 1977), soil washing (Newell 1971; Bishop 1977), Vagvolgyi flotation (Mason 1970), and the cold water process (CWP) (South 1964). Dry-sifting fresh soil and litter is often impossible if the sample contains much water; Bishop (1977) recommended soil washing in that instance. Soil washing was 63-100% efficient

for recovering slugs from samples (South 1964; Hunter 1968), but an abundant supply of running water is necessary for the technique. Also, both dry sifting and soil washing are extremely laborious. Extraction of snails from soil samples by the Vagvolgyi technique is quicker; Mason (1970) determined it to be 84% efficient. However, all snails are killed in the process and slugs are not recovered. The CWP is quick, requires little water, and is 81-99% efficient at recovering slugs in all but packed soil types (South 1964; Hunter 1968). This technique has not been used for recovery of snails until the present study.

Tests were conducted to determine the efficiency of the CWP at recovery of terrestrial snails. The CWP was a much quicker technique than dry sifting and more snails were recovered. Decreased recovery of live snails by sifting was probably due to decreased visibility of snails when mixed within leaf litter. The relative proportions of snail species recovered were approximately the same with both techniques, so species-specific differences in response to rising water may be discounted. The efficiency of recovery of known numbers of *D. cronkhitei* and *V. gracilicosta*, ranging from 60-100%, was similar to that reported for slugs using the same technique.

It is a common belief that terrestrial gastropods cannot survive submergence under water. This belief is illustrated in Boycott's (1934) statement that, "most land snails and slugs are easily drowned. If they fall into water

their mucus is so diluted that they cannot crawl out. Heavy rain, apart from the impact of the drops, may be quite destructive and they do not usually go out into it". Many species of terrestrial snails and slugs can no doubt survive extended periods of submergence. In the present study, 100% of *D. cronkhitei* and *V. pulchella* survived 24 hours of submergence and were observed crawling on the vial walls.

The CWP appears to be a rapid and efficient method of obtaining quantitative information on the distribution and abundance of terrestrial gastropods, at least in northern temperate regions. In tropical habitats, where many gastropod species have arboreal distributions (Peake 1968), additional methods may be required.

Species of terrestrial gastropods recovered in the present study have been reported previously from Alberta by Platt (1980), Van Es and Boag (1981) and/or Boag and Wishart (1982). Of the four species infected with *P. boughtoni* larvae, one (*Euconulus fulvus*) was of the family Zonitidae, species of which are not commonly reported as intermediate hosts of *Protostrongylus*. Since only one of 356 *E. fulvus* was infected, this species does not appear to be a normal intermediate host of *P. boughtoni*. Although Pillmore (1956) regarded *E. fulvus* as refractive to infection with *Protostrongylus stilesi* and/or *Protostrongylus rushi*, Forrester (1971), Latson (1977) and Boag and Wishart (1982) found that this species was infected with a *Protostrongylus*-type larva on bighorn sheep range. The

possibility exists that those larvae were *P. boughtoni* or *Orthostrongylus macrotis*.

Although all larvae recovered in the present study were designated as *P. boughtoni*, since mule deer (*Odocoileus hemionus*) and white-tailed deer (*Odocoileus virginianus*) occurred in the study area, it is possible that some larvae were actually *O. macrotis* (See comparison, Chap. I). This is unlikely because deer feces were never encountered in pellet count quadrats, indicating that deer seldom frequented my transect sites. Also, Stock (1978) reported that only 8% of mule deer and 3% of white-tailed deer from Alberta were infected with *O. macrotis*. Moose (*Alces alces*), frequently observed in the study area, may also have been infected with *O. macrotis*, but the parasite is not common in them (Samuel *et al.* 1976).

Vertigo ovata and *Columella edentula* are not considered important in transmission of *P. boughtoni* on the study area because of their smaller, spatially-restricted populations. *V. gouldi* was found in all habitat types including ST, where the acid nature of the substrate likely precluded the establishment of most other species. *V. gouldi* appears to be very much a generalist in habitat requirements, an observation supported by Van Es and Boag (1981) who found it on 90% of sites sampled in central Alberta, and Boag and Wishart (1982) who recovered it from all habitats sampled on bighorn sheep range in southwestern Alberta.

For transmission of *P. boughtoni* to occur, an infected snail must be ingested by a snowshoe hare. In the present study, there were no readily-identifiable "foci" of infection in the sense of Lankester and Anderson (1968) and Platt (1978). In those studies the definitive hosts were spatially concentrated, in the former instance on an island, and in the latter on a townsite. Such concentration would amplify transmission in those areas. *V. gouldi*, the primary intermediate host species for *P. boughtoni*, was relatively common in each habitat type. Since neither the prevalence nor intensity of infection varied among habitat types, the potential for transmission was largely dependent on relative abundance of *V. gouldi*, a variable which could differ by a factor of approximately 10.

Prevalence of *Parelaphostrongylus* spp. in slugs fluctuates seasonally, due in part to an annual die-off of older, heavily-infected individuals and subsequent replacement in samples by young recruits to the population (Lankester and Anderson 1968; Platt 1978). The monthly size distribution of *V. gouldi* indicates a more stable population with reproduction occurring over most of the summer and no catastrophic die-off of older animals. The stability of *P. boughtoni* infection in *V. gouldi* may be related to the stability of the snail populations. The life span of *V. gouldi* is unknown, but is likely greater than one year.

Lankester and Anderson (1968) noted similar stability in prevalence of infection with *Parelaphostrongylus tenuis*

in *Zonitoides nitidus*, possibly indicating that age structure of the snails was relatively constant. Lankester and Anderson (1968) and Platt (1978) noted increases in prevalence of *Parelaphostrongylus* spp. infection with slug size (=age). Rodonaya (1977) noted a similar prevalence increase in *Helicella derbentina* infected with *Protostrongylus tauricus*. No such increase was noted in the present study. Snails of the smallest infected size class had the same prevalence and intensity of infection with *P. boughtoni* as the largest class, and this may indicate that only the smallest snails were susceptible. Cabaret (1981) determined that previous exposure of various species of land gastropods to protostrongylid larvae may reduce their receptivity on subsequent reexposure, although Lankester and Anderson (1968) found no evidence of this. Also, L3's may emerge from infected snails (Chap. I), and if this were a common occurrence, it could prevent an increase in both the prevalence and intensity of infection among older snails. Another possible reason for the unchanged prevalence and intensity in older snails may be an increased rate of mortality among infected individuals.

Larvae of *P. boughtoni* must overwinter in *V. gouldi*, since they were found in the earliest collections from the second week in May. The soil in most of the habitats was still frozen just below the surface in the third week of April. Even if larvae were to enter snails some time in early April, low temperatures would retard their development

(Gerichter 1951) and none would be infective until late May. Overwintered L3's were available to snowshoe hares as soon as the snails became active.

Prevalences reported for gastropods infected with metastrongyloid larvae vary widely, no doubt partly in relation to the objectivity with which the gastropods were collected. Pillmore (1955) collected *Pupilla muscorum*, by field search and sifting techniques, from bighorn sheep bedding areas at Buffalo Peaks, Colorado in 1954 and found 2.6% were infected with 1-25 *Protostrongylus* larvae. In 1955, using a more random method of sampling over a wider area of sheep range, Pillmore (1956) found prevalence of only 0.5%. Latson (1977) collected snails by field searching from bighorn sheep bedding grounds on Pikes Peak known to contain infected snails. 10.7% of *Pupilla*, 6.7% of *Vertigo*, and 16.7% of *Euconulus* (12 collected) were infected with 1-40 *Protostrongylus* larvae. Within the USSR, Rizhikov *et al.* (1956a) collected molluscs by field searching and found 30% of *Vallonia tenullabris* and 36% of *P. muscorum* infected with *P. kamenskyi* and/or *P. pulmonalis* larvae. Rodonaya (1977) found 10.1% of all *Helicella derbentina* collected were infected with 1-22 *Protostrongylus tauricus* larvae.

More objective collection of gastropods was practiced in the work on related protostrongylids by Lankester and Anderson (1968) and Platt (1978). In the former study, prevalence of infection with *P. tenuis* larvae in susceptible species of molluscs ranged from 0.1 to 8.2% with an

intensity range of 1-97. In the latter, 0.7-3.97% of susceptible species were infected with 1-377 *P. odocoilei* larvae. The higher intensities reported within intermediate hosts of *Parelaphostrongylus* species resulted when relatively larger intermediate hosts were collected in areas of optimal parasite transmission ("foci").

None of the mentioned studies are wholly comparable with the present work due to differences in snail collection methods. Direct collection of gastropods in the field is suited only to large, conspicuous species and under-represents young and concealed individuals (Mason 1970). Many objections have been raised to baiting and trapping methods (South 1964; Hunter 1968; Newell 1971; Bishop 1977; Beyer and Saari 1978). However, Bishop (1977) felt that baiting and trapping methods may be of value in detecting rare or secretive species and Boag (1982) stated that permanent trap plots allowed more accurate temporal population comparisons.

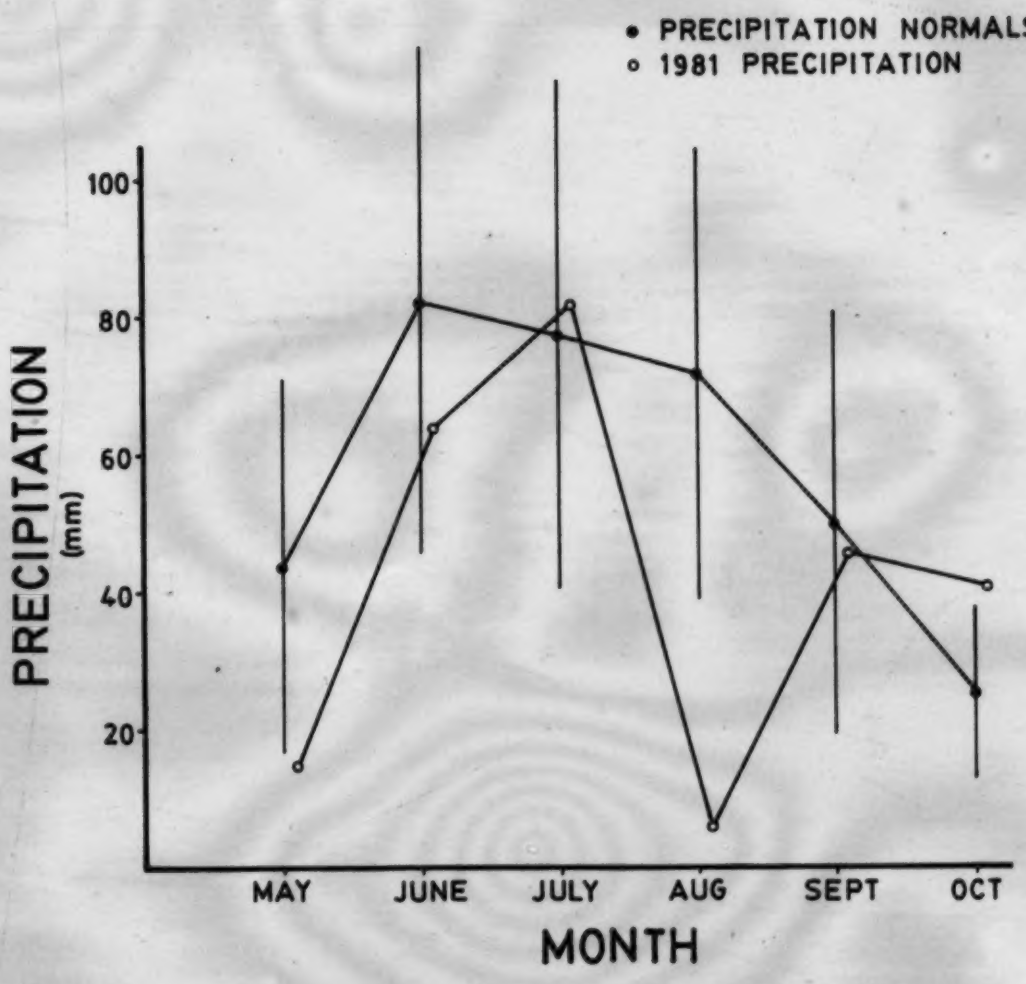
It appears that the percentage of randomly-sampled gastropod intermediate hosts infected with wild-mammal source protostrongylid larvae is usually less than 10. Selectively sampling areas of frequent definitive host use will increase the prevalence, and data on such "foci" should be presented separately for clarity. Numerous studies exist in which the prevalence of infection with protostrongylid larvae was determined for snails on domestic livestock range (e.g. Gerichter 1951; Matekin 1954; Panin 1964; Urban 1980;

Cabaret 1981; numerous others). Variation in husbandry methods such as timing of pasturage and anthelminthic treatment would so affect infections in snails as to prevent comparison with similar wild animal species of parasites.

Few terrestrial gastropod studies have attempted to produce an absolute value for snail density, and collection methods are far from standard. Mason (1970) determined that densities of the pupillids *Columella edentula* and *Pupilla muscorum* in an English beech forest were 3.37/m² and 0.67/m² respectively. *Vertigo gouldi* is normally quite abundant in Albertan forest habitats (Van Es and Boag 1981; Boag and Wishart 1982) and shows a highly aggregated distribution (Boag and Wishart 1982) in common with many other species of terrestrial gastropods (Mason 1970).

Population stability may be a common characteristic among snails with a long breeding season, and a relatively long lifespan (1.5-3 yr) (Mason 1970). On three of five habitat types in the present study, density of *V. gouldi* was temporally stable. On SS and HW, populations declined from June until August, with subsequent recovery of numbers on SS only. Berry (1966) found that whereas frequent and heavy rain promoted reproductive activity and survival of young and adult gastropods, dry spells had the opposite effect. Rainfall from the end of July to the beginning of September was well below normal for the area (Fig. 37). Although this drought cannot account for the initial density decline in July, it may be responsible for exacerbating the trend.

Figure 37. Precipitation measured at Slave Lake, Alberta from May to October, 1981, and precipitation normals for those months. Bars above and below normals represent one standard deviation. (Data Sources: Monthly Record, Meteorological Observations in Western Canada. Vol. 66, No. 5-10, Part 1; and, Canadian Climate Normals. Precipitation. Vol. 3, 1951-1980. Both volumes from Atmospheric Environment Service, Environment Canada.)


• PRECIPITATION NORMALS
○ 1981 PRECIPITATION
PRECIPITATION (mm)
100
80
60
40
20
MAY
JUNE
JULY
AUG
SEPT
OCT
MONTH

Precipitation returned to near normal in the second half of September, coinciding with the increase in snail density on SS.

Drought would have a great effect on SS transects which were normally quite dry. The herb layer was very sparse and mainly composed of bryophytes (Appendix IIIc). The amount of ground covered by shrubs was also very low. Thus moisture would be rapidly depleted from the litter layer. Although the substory vegetation on the HW transects was normally quite dense and would no doubt aid retention of soil moisture under normal conditions, attacks of tent caterpillars in late May and June defoliated the overstory trees and many of the understory shrubs. Direct sunlight and wind likely desiccated the substrate and may have caused the reduction in snail numbers.

Density of *V. gouldi* increased on SS transects through September and attained near-spring levels in October, implying that many snails retreated deeper into the substrate in the dry weather, but returned to the surface at the onset of more favourable conditions. Densities on HW showed no such rebound, suggesting that substrate desiccation induced greater mortality in those populations.

Reports of very high levels of *Protostrongylus* infection in leporids are not uncommon (Table XI). Intensities of *P. boughtoni*, where given, tend to be much lower than the findings of the present study. Detection and recovery of all *P. boughtoni* in lungs is a difficult and

TABLE XI. PREVALENCE AND INTENSITY OF *PROTOSTRONGYLUS* INFECTIONS IN WILD POPULATIONS OF LEPORIDS

SPECIES	HOST	LOCATION (reference)	PREVALENCE %	INTENSITY $\bar{x}$ (range)
P. boughtoni	*Lepus americanus*	Alberta (This Study)	92.3	27.3 (1-284)
"	"	Alberta (Shostak unpub.)	53.0	5.0 (1-65)
"	"	Alberta	52.0	
		Alaska	65.0	
		New Brunswick (Tobon & Yuill unpub.)	60.0	
"	"	New Brunswick	11.0	
		New York (Goble & Dougherty 1943)	41.0	
"	"	New York (Goble & Cheatum 1944)	87.0	
"	"	Quebec (Appendix VI)	100	16.4 (4-54)
"	"	Michigan (Bookhout 1971)	44.3	6.1 (?-37)
"	"	Minnesota	8.0	
		Minnesota & Wisconsin (Green & Shillinger 1935a,b)	62.0	
"	"	Manitoba (Boughton 1932)	83.3	"up to 50-60/lobe"
"	"	Colorado (Olsen 1954)	100	
P. sylvilagi	*Sylvilagus nuttali*	Wyoming (Scott 1943)	90.0	
P. pulmonalis/ *P. kamenskyi*	*Lepus timidus*	Yakutsk SSR (Kontrimavichus & Popov 1960)	96.6	59-565
P. tauricus	*Lepus europaeus*	Crimea SSR (Shults and Kadenatsii 1949)		"often 100, to 8250"
"	"	Crimea SSR (Kadenatsii 1969)	65-100	

time-consuming task and most researchers use more rapid recovery methods than tissue teasing, such as pressing lung tissue between glass plates to examine with transmitted light. In my experience, such techniques are less efficient and result in lower values of prevalence and intensity. Another consideration results from the observation that many of the reports originate from the southern edge of geographical distribution for *Lepus americanus*. Dogiel *et al.* (1964) stated that hosts at the edge of their range should have lower prevalence of infection, and lower intensity infections, than those centrally located.

Goble and Cheatum (1944) provide the only report of differences in infection of *L. americanus* with *P. boughtoni* due to age: 91% prevalence in adults and 77% in juveniles. The month of collection was not given, although it was likely in mid-summer. They also found, with a small sample size, that all infected juveniles were older than 35 days while negatives were about 28 days of age. With a larger sample collected, I found infected juveniles at a minimum age of 23 days and patent infections at a minimum age of 40 days. Given a prepatent period in the hare of 25 to 27 days (Chap. I), then some juveniles might begin ingesting infected snails at approximately two weeks of age, long before they would normally be weaned (Rongstad and Tester 1971). However, if a juvenile were separated from its mother at 2 weeks of age, it could survive weaning (Bursey 1977; pers. obs.); neonatal hares have been observed nibbling on

lettuce within a week of birth.

Intensity of infection with certain nematode parasites in *Oryctolagus cuniculus* was shown to differ between males and females during the breeding season, possibly due to hormonal influences (Bull 1964; Dunsmore 1966). A similar pattern was observed with *Obeliscoides cuniculi* infections in *L. americanus* from Maine (Gibbs *et al.* 1977). Variation in recruitment between males and females is another possible reason for the numerous reports of this phenomenon among parasites (Esch *et al.* 1977). These factors were apparently without similar influence in the present study. Erickson (1944) also found no sex-related difference in prevalence of 6 helminths infecting snowshoe hares.

A monthly prevalence of infection pattern similar to that of *P. boughtoni* was reported by Kontrimavichus and Popov (1960) for infections of *P. kamenskyi and/or P. pulmonalis* in *Lepus timidus* from Yakutsk. Prevalence was near 100% for most of the year with a reduction from May until June, possibly corresponding with the period of recruitment of young, uninfected hares into the population. Intensity exhibited a pattern opposite to the present study, peaking from January to February and reaching lowest levels from August to September. Increased immunological competence resulting from high quality summer forage was presented by way of explanation for lower rates of establishment of adult worms. Under such conditions, many larvae which invaded the hosts were arrested in an early stage of development by a

tissue response. The decline in fodder quality brought on by cold weather was thought to lower resistance of the hares, allowing an increased number of larvae to mature, resulting in highest intensities of infection at a time when transmission could not occur due to snow cover of snails.

Arrested development is reported to occur with many nematodes, most often species with free-living larvae. Three major groups of factors are believed to induce developmental arrest (Schad 1977): environmental (temperature, humidity), parasite (crowding effect), and host (immune response). In the present study, fourth-stage larvae were found in the lungs of adult hares in greatest abundance during the winter months. Similarly, Gibbs *et al.* (1977) found that the abundance of arrested L4's of *O. cuniculi* in snowshoe hares peaked from October to February. Since direct transmission of *P. boughtoni* is unlikely (Chap. I), L4's found were likely ingested before snow covered the intermediate hosts, and thus developmental arrest is indicated. I have no evidence to suggest that the L4 is the arrested stage, but most reports of arrested larvae do implicate it (Schad 1977).

Although development of some larvae ingested in the summer and fall may have been arrested due to a relatively strong immune response, the possible weakening of that response in the winter did not result in establishment of massive numbers of *P. boughtoni*. If adult *P. boughtoni* have a relatively short lifespan as indicated by infections in

lab-reared naive hares, then possibly reactivated larvae replaced expired adults only, resulting in a fairly constant intensity over the winter months. A similar population-regulating function of arrested development was proposed by Michel (1974) who compiled evidence that gastro-intestinal nematode populations in grazing animals are constantly turned over. Adult nematodes which are lost from the population are replaced by larvae arrested in somatic tissue.

Considering the relative lack of resistance to *P. boughtoni* infection displayed by juvenile hares, the infrapopulations of lungworms within adults appear to be regulated within very narrow limits. The decline in intensity shown by the first and second litter groups is evidence that partial immunity to reinfection might develop in the hares after an initial heavy infection. A similar age-related pattern was apparent in infections of *O. cuniculus* with *Trichostrongylus retortaeformis* and *Taenia pisiformis* in New Zealand (Bull 1964). Incidence increased with age, but intensity quickly peaked and in some instances declined, indicating an acquired resistance to reinfection.

Dineen (1963) proposed the existence of an immunological threshold value for a parasite burden within a host, beyond which the host responds to reduce the population. This may be accomplished variously by expelling all or part of the population, reducing reproductive output, or retarding larval development. Subsequent research on

trichostrongyles (Donald *et al.* 1964) supported the basic hypothesis and provided evidence that retarded development of larvae was the major mediating factor. Dunsmore (1961) demonstrated that immunosuppression decreased the proportion of arrested *Ostertagia* spp. in infected sheep. Acquired immunity has been shown to induce developmental arrest in the lungworm *Dictyocaulis viviparus* (Michel 1974), indicating that these mechanisms are likely not restricted to gastro-intestinal nematodes.

The possibility exists that some hares have innate resistance to *P. boughtoni* infection, and the fall decline in juvenile mean intensity was due to a die-off of susceptible individuals. A possible genetic resistance to experimental infection was detailed in Chapter I (S.H. 28's offspring). It was not possible to detect such resistant hares with the methods of the present study as individual intensity of infection varied so greatly over time, and individual hares were sampled only once.

If larval output from bighorn sheep infected with *P. stilesi* and/or *P. rushi* reflects the number of adult worms in the lungs, then the seasonal dynamics of infection with these lungworms are more similar to those of the *Lepus timidus* lungworm species (Kontrimavichus and Popov 1960). Uhazy *et al.* (1973) and Gates (1975) documented peak larval output in February and March, and minimum output in mid-summer, for bighorn sheep herds in the Canadian Rockies. An often-repeated hypothesis for such "winter peaks" in

larval output is that transmission to terrestrial gastropods is facilitated by flooding the environment with the resistant first-stage just before snails become available for exposure (Samuel 1978; Boag and Wishart 1982). The absence of a "winter peak" in the present study may be due to differences in many factors including hosts, habitat and climate.

The exponential increase in intensity demonstrated by each juvenile group indicated that infective larvae were available at a consistently high level of abundance from the time the first group became present, and that infrapopulation size was apparently not controlled by the parasite factor of crowding (Schad 1977). Also, this is evidence for the absence of a peak transmission period with respect to infected intermediate hosts, a characteristic indicated by data from the previous section which showed a stable level of *V. gouldi* infection.

The number of larvae in the lungs or feces of a juvenile snowshoe hare is a relatively good predictor of the intensity of *P. boughtoni* infection. The lung count is a more accurate predictor, as may be expected. Forrester and Senger (1964) found that there was a poor relationship between fecal larval output and intensity of infection for bighorn sheep with *P. stilesi* and/or *P. rushi* unless samples were compared on a herd basis. Pillmore (1961) and Uhazy *et al.* (1973) found similar relationships. All lungs from the first-mentioned study were from sheep greater than one year

old, and the data are more comparable to that for adult snowshoe hares, which showed a poor relationship between larvae in lungs and feces and adult *P. boughtoni* in the lungs. In many instances, infections in the lungs resulted in no output of larvae in the feces, even with relatively high intensities. The relationship of lung larvae to adult nematodes was much more consistent, though not as good as the juvenile hare relationship.

The poor relationship between numbers of first-stage larvae and adult nematodes in the lungs of adult snowshoe hares is evidence of suppressed reproduction due to a host immune response. This response may also be responsible for regulating the intensity of infection within adult hares at a level much lower than that of juvenile hares. Reproductive suppression in adults and not juveniles indicates that the immunity is acquired after initial infection with *P. boughtoni*.

The diaphragmatic lobes were the preferred site of establishment for *P. stilesi* in bighorn sheep (Pillmore 1961; Forrester and Senger 1964), a preference also shown by *P. boughtoni*. Lesions were occasionally found on apical, cardiac and intermediate lobes. Forrester and Senger (1964) and Uhazy and Holmes (1971) determined a preference for the right side of the lungs, a characteristic not seen in the present study. Possible explanations for diaphragmatic lobe preference by *Protostrongylus* include nutritional advantage, reproductive suitability and original site of entry into the

lungs. Relative ventilation and blood perfusion may be greater in diaphragmatic than apical lung lobes in mammals (Amis *et al.* 1982), possibly providing an optimal environment for the lungworms. A simpler explanation may be that lobes acquire nematodes in direct proportion to their relative volumes, resulting in highest populations in the diaphragmatics.

There was no evidence of transplacental transmission of *P. boughtoni* in hares trapped on the study site. Observations by Goble and Cheatum (1944), Tobon and Yuill (unpubl.) and others of *P. boughtoni* infections in very young hares prompted speculation that lungworms may be acquired *in utero*. I believe this is unlikely in light of my digestion data (Appendix II) and also my evidence for early acquisition of infective larvae by ingestion of snails. The conditions for transmission of L3's from snail to host in the sheep-lungworm systems where prenatal infection has been demonstrated are likely very different from those in the snowshoe hare-lungworm system at the Slave Lake study area. Azimov and Kulmamatov (1978) induced transplacental transmission of protostrongylids in domestic sheep by exposing pregnant ewes to 3,500-4,000 L3's, an inoculum likely much greater than that which an individual pregnant hare would possibly ingest. Transplacental transmission of *P. boughtoni* is not likely a factor in the snowshoe hare-lungworm system.

Fecal pellet counts as an estimate of relative activity or habitat preference have gained some acceptance in studies of rabbits and hares (Taylor and Williams 1956; Miller 1968; Bartholemew 1970; Jaksii *et al.* 1979; Rogers and Myers 1979,1980; Wolff 1980). The technique has long been used in studies of wild ruminants, and results obtained are commonly applied to management programs (Neff 1968). Although widely accepted, all pellet count techniques are underlain by a number of assumptions.

In the present study, I assumed that the density of pellets within a habitat type reflected the amount of feeding in that area by snowshoe hares. Miller (1968), relying on the same assumption, admitted that there were no data available to correlate fecal deposition with the amount of grazing by hares. Neff (1968) concurred with the statement that, "the relationship between defecation and other animal activities remains conjectural". Snowshoe hares have crepuscular and nocturnal activity patterns (Bider 1961). During the day, soft feces are produced for reingestion (Banfield 1977; pers. obs.). Thus it seems reasonable to assume that hares deposit hard feces in areas where they forage during the evening and at night. Counts of hard pellets should produce a reasonable estimate of relative feeding activity between habitat types unless certain habitats are used for specific non-feeding activities.

The random-quadrat approach is preferred for quantitative estimates of pellet densities. However, many authors recommend removal of all pellets from permanently-designated plots some time before counts are to be made (Miller 1968; Neff 1968; Wolff 1980). Removal of pellets avoids over or underestimation of habitat use due to change in seasonal habitat preference. Also, relative volumes of leaf fall in different habitat types will influence the numbers of pellets counted. I felt that total pellet removal from all plots would have been impractical as there were up to 200 pellets on each of the 250 quadrats sampled monthly. In areas with heavy undergrowth, such an activity would have greatly disturbed the habitat and affected potential use by hares. Therefore pellets were not removed, and the resulting data must be viewed with caution due to possible biases.

Wolff (1980) documented changes in snowshoe hare habitat usage between winter and summer by live trapping and pellet counting. In the winter, more hares were found in areas of dense vegetation than in the open plots. In summer, all areas were utilized. However these data were collected during the low phase of the population cycle. Distribution was relatively even in 1972 at the beginning of the decline phase when hare densities were still high. This was consistent with Keith's (1966) conclusion that, "hares occupy the most favorable habitat at low population densities and disperse into less favorable areas as their

numbers increase". Also, highest numbers of dispersing hares and highest dispersal rates were recorded during a population peak (Windberg and Keith 1976). Thus an annual change in habitat usage may be prevented during years of peak population density by near-total occupancy of most habitats.

The present study was undertaken during the apparent peak of the local snowshoe hare population cycle. Thus a seasonal habitat change was unlikely and probably did not affect the pellet densities on transects. However, relative volume of leaf fall must have differed between primarily coniferous and deciduous habitat types resulting in an overestimate of usage of the former, and an underestimate of the latter. The actual extent of bias is unknown but likely small, because only in HW habitats was a significant proportion of the substrate area obscured by leaf fall.

ST and SS habitats consistently had the highest pellet densities. This may have reflected the quality of cover from predators afforded by these two areas as ST was most commonly recognized for dense growths of black spruce and labrador tea, and SS had a dense canopy of heavily-branched conifers. However, Wolff (1978) found spruce in 80-100% of Alaskan snowshoe hare stomachs examined through the year, and determined that it comprised 15% of the summer diet. With reference to the literature, he concluded that conifers were a major constituent of snowshoe hare diet throughout North America. Forage plants were no doubt more abundant in

the secondary growth of CUT habitats. The attraction of food in this habitat may have been offset by the relative reduction in cover on some of the more recently disturbed plots, resulting in lower pellet densities compared with the primarily-coniferous habitats. Relatively less usage of the MW plots was probably a result of both lower forage availability and thinner cover. For similar reasons, HW plots showed the least usage by hares. Relative paucities of food and cover in HW were further aggravated by destruction of vegetation by tent caterpillars. Leaf fall may have hidden a significant proportion of pellets in this habitat, although this is not indicated in comparisons of counts taken in spring and fall.

Decrease in pellet densities in all habitat types throughout the period of study may have been a result of the decay of pellets produced and accumulated in winter. Pellets produced in winter would rapidly freeze and be subject to little decay until the spring thaw. After the snow cover disappeared, most of these pellets would begin to decompose at approximately the same time, producing the gradual downward trend in density observed. If only pellets produced during the snow-free seasons were counted, the densities might be more stable or even increase due to recruitment of young hares into the population.

CONCLUSIONS - TRANSMISSION OF PROTOSTRONGYLUS BOUGHTONI

Efficient transmission of a parasite within a host population is central to the survival and perpetuation of that parasite's own population. Thus the pressure of natural selection may be expected to increase transmission from a random-chance occurrence to one of increased probability of host-parasite contact. This is often accomplished by improving the synchrony of parasite and host life cycles to accentuate contact between populations. However, parasite populations determined solely by transmission are inherently unstable, though such systems may occur under special conditions (Bradley 1974). Stabilizing mechanisms evolve in most host-parasite systems where transmission occurs in excess of that required for continued existence of the parasite suprapopulation. In this chapter I will summarize details of *Protostrongylus boughtoni* transmission among host populations on my study area, as well as speculate on the stability of that system over time and the adaptability of the present methods to future research on related nematodes of the Metastrongyloidea.

In parasitic systems where there are multiple intermediate and/or definitive hosts, often a single host species is of primary importance in perpetuation of the parasite population, and it is in that species that density-dependent regulation of the parasite population is likely to occur (Holmes *et al.* 1977). Identification of such primary hosts can best be achieved by calculation of

relative rates of flow of parasites through each host population utilizing data on the temporal dynamics of infection in each of the species (Holmes *et al.* 1977). The value of this method was demonstrated by Leong (1975) in an analysis of *Metechinorhynchus salmonis* in fish from Cold Lake, Alberta, and by Kontrimavichus and Atrashkevich (1982) in a study of five species of acanthocephalans in birds. A simple, non-flow model was constructed by Maklakhova (1979) to identify habitats in which the probability of hares and elk (*Alces alces*) acquiring protostrongylid infection was greatest.

Transmission models were constructed for the *P. boughtoni* system using data from the preceding chapter, snail and hare data not presented in previous chapters, and in some cases, data from published sources to answer the following questions: 1) which snail host was responsible for the greatest amount of parasite flow to the snowshoe hare population? 2) which snail host received the greatest flow of first-stage larvae? 3) in which habitat did the greatest amount of snail to hare parasite flow occur? 4) in which habitat did the greatest amount of hare to snail flow occur? 5) which age group of hares contributed the greatest flow of first-stage larvae to the system? and 6) in which month did the greatest flow of first-stage larvae to the environment occur? Similar to Leong's (1975) study, parasite flow rates were not actually measured, but were deduced from a static picture of distribution of *P. boughtoni* in the various snail

species.

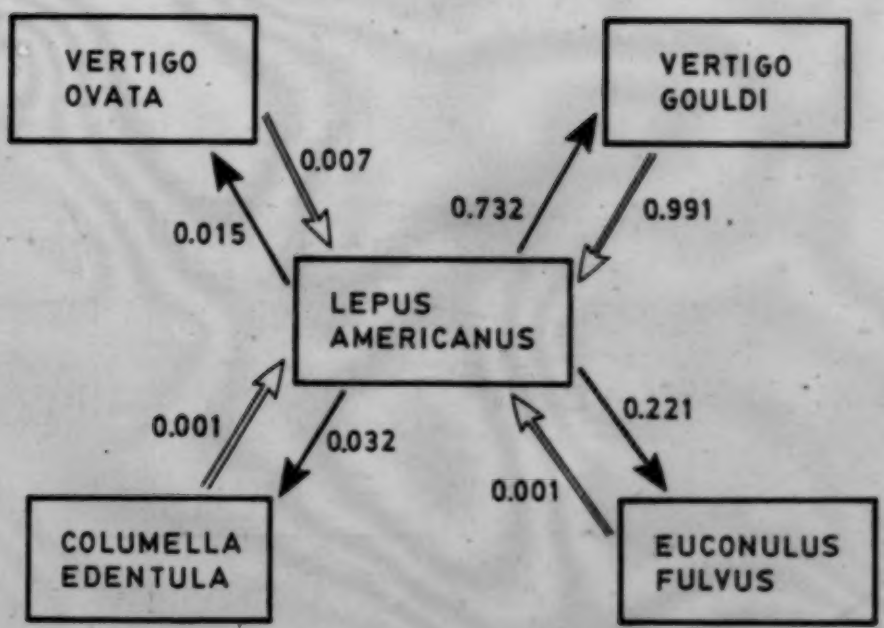
In Chapter II, detailed examination of *P. boughtoni* infection within intermediate hosts was restricted to *Vertigo gouldi* in recognition of its larger population relative to other snail hosts. However, the locations of snail species relative to hares must be considered in any assessment of parasite flow. Calculations of snail to hare and habitat to hare flow were organized in a four by five matrix with the four intermediate host species assigned to rows, and the five habitat types assigned to columns (Table XII). In each cell, the mean density of snails per m^2 on the habitat type, the abundance of *P. boughtoni* (prevalence times mean intensity) in the snail species, the mean density of hare fecal pellets per m^2 on the habitat type, and the habitat type proportion of the total study area were multiplied. All cell products of a row were summed, as were all cell products of a column. Each row sum was divided by the grand sum to calculate the relative flow of *P. boughtoni* to hares from that snail species (Fig. 38). In order to determine the relative flow of *P. boughtoni* to hares occurring in each habitat type (Fig. 39), each column sum was divided by the grand sum.

Algebraic examples of these calculations are as follows. If the variables for snail A in habitat type A are snails/m^2 = a, *P. boughtoni* abundance = b, pellets/m^2 = c, habitat proportion = d, then the product for this cell would be p^1:

TABLE XII. CALCULATIONS OF *PROTOSTRONGYLUS BOUGHTONI* FLOW FROM SNAILS TO HARES AND FROM HABITAT TYPES TO HARES

SNAIL SPECIES	HABITAT TYPE					SUM
	ST	SS	MW	CUT	HW	
Vertigo gouldi						
Snails/m^2	5.87	53.33	28.81	24.93	9.60	
P.b. Abundance	4.44	9.02	7.35	4.41	10.95	
Pellets/m^2	37.42	34.48	15.51	29.54	7.70	
Habitat Prop.	0.24	0.117	0.352	0.08	0.203	
Product	234.06	1940.58	1156.07	259.81	164.31	3754.83
Vertigo ovata						
Snails/m^2			1.06			
P.b. Abundance			4.55			
Pellets/m^2			15.51			
Habitat Prop.			0.352			
Product			26.33			26.33
Columella edentula						
Snails/m^2				1.20		
P.b. Abundance				1.92		
Pellets/m^2				29.54		
Habitat Prop.				0.08		
Product				5.44		5.44
Euconulus fulvus						
Snails/m^2	0.93					
P.b. Abundance	0.28					
Pellets/m^2	37.42					
Habitat Prop.	0.24					
Product	2.34					2.34
SUM	236.40	1940.58	1182.40	265.25	164.31	
GRAND SUM	3788.94					

Figure 38. Relative flow rates of Protostrongylus boughtoni between snails and snowshoe hares in the study area. Solid arrows indicate flow of first-stage larvae from the definitive host to intermediate hosts. Outlined arrows indicate flow of third-stage larvae from intermediate hosts to the definitive host. Values indicate proportions of flow.


VERTIGO OVATA
VERTIGO GOULDI
LEPUS AMERICANUS
COLUMELLA EDENTULA
EUCONULUS FULVUS
0.007
0.015
0.732
0.991
0.001
0.032
0.221
0.001

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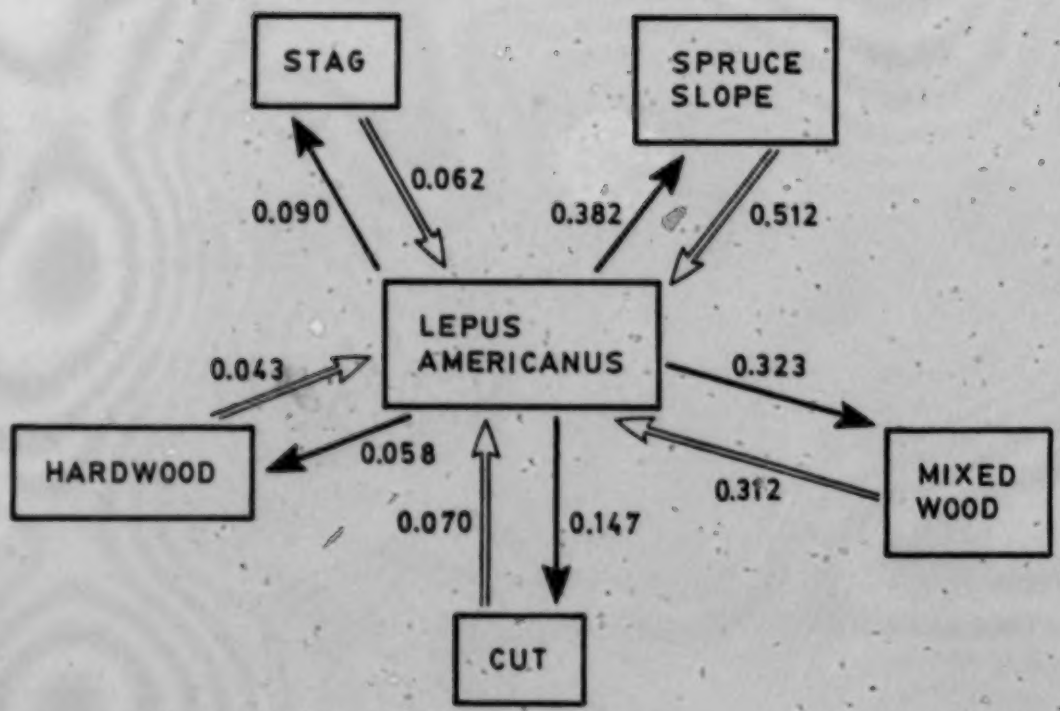
STAG
SPRUCE SLOPE
0.062
0.090
0.382
0.512
LEPUS AMERICANUS
0.043
0.323
HARDWOOD
0.058
MIXED WOOD
0.312
0.070
0.147
CUT

$$a \times b \times c \times d$$

$$= p^1$$

Summation of five cell products of a row would produce the row sum, pr^1:

$$p^1 + p^2 + p^3 + p^4 + p^5$$

$$= pr^1$$

Summation of four row sums would produce the grand sum, prt:

$$pr^1 + pr^2 + pr^3 + pr^4$$

$$= prt$$

Division of the first row sum by the grand sum would produce the proportion of flow from snail species A in the study area, ppf^1:

$$pr^1/prt$$

$$= ppf^1$$

Several assumptions are inherent in these calculations. One is that third-stage larvae (L3's) from all snail species were equally infective. Another is that there was no differential exposure of infected snails to hares due to normal or parasite-altered host behavior. Assumptions inherent in the pellet count technique were detailed in Chapter II.

Over 99% of *P. boughtoni* flow to hares on the study area was from *V. gouldi*, and the other intermediate hosts were of minor importance in transmission (Fig. 38). The constant temporal abundance of *P. boughtoni* in this host (Chap. II) might indicate that a density-dependent form of parasite population regulation was operating. Possible

mechanisms to explain the unchanging levels of *P. boughtoni* in *V. gouldi* include increased mortality of heavily-infected individuals, and larval emergence due to a "crowding effect". Immune regulation was less likely as the necessary complexity of response has not generally been noted in the invertebrates (Lackie 1980). Consideration must also be given to the possibility that seasonal variation in parasite abundance may not have been detected due to the relatively small sample size of infected snails (44 in six months).

The second flow diagram (Fig. 39) shows that more than half of all parasite flow from snail to hare occurred in the Spruce Slope habitat. Even though this habitat type was not very abundant, it had the highest density of *V. gouldi* and the second highest density of fecal pellets. Due to temporal variation in the density of *V. gouldi* in the SS habitat (i.e., populations were depressed in mid-summer, Chap. II), transmission rates may have been relatively higher in the early spring and late fall than in mid-summer. Parasite flow to hares was also very high in Mixed Wood. Stag, Cut, and Hardwood were of minor importance in this aspect of transmission.

The relative flow of first-stage larvae (L1's) to each intermediate host species from hares, regardless of actual susceptibility to infection, and the flow of L1's to all potential snail hosts in each habitat type were calculated using a matrix design similar to that mentioned above (Table XIII). Differences were the exclusion of parasite

TABLE XIII. CALCULATIONS OF *PROTOSTRONGYLUS BOUGHTONI* FLOW FROM HARES TO SNAILS AND FROM HARES TO HABITAT TYPES

SNAIL SPECIES	HABITAT TYPE					SUM
	ST	SS	MW	CUT	HW	
Vertigo gouldi						
Snails/m^2	5.87	53.33	28.81	24.93	9.60	
Pellets/m^2	37.42	34.48	15.51	29.54	7.70	
Habitat Prop.	0.24	0.117	0.352	0.08	0.203	
Product	52.72	215.14	157.29	58.91	15.01	499.07
Vertigo ovata						
Snails/m^2			1.06	1.86		
Pellets/m^2			15.51	29.54		
Habitat Prop.			0.352	0.08		
Product			5.79	4.40		10.19
Columella edentula						
Snails/m^2		1.86	1.46	1.19	2.39	
Pellets/m^2		34.48	15.51	29.54	7.70	
Habitat Prop.		0.117	0.352	0.08	0.203	
Product		7.50	7.97	2.81	3.74	22.02
Euconulus fulvus						
Snails/m^2	0.93	9.43	9.03	14.60	13.28	
Pellets/m^2	37.42	34.48	15.51	29.54	7.70	
Habitat Prop.	0.24	0.117	0.352	0.08	0.203	
Product	8.35	38.04	49.30	34.50	20.76	150.95
SUM	61.07	260.68	220.35	100.62	39.51	
GRAND SUM	682.23					

abundances, and data on mean densities of non-infected, potential intermediate hosts were included. Row calculations, performed as in the previous example, resulted in the relative flow of L1's from hares to each species of intermediate host (Fig. 38), and column calculations produced the relative flow of L1's to all intermediate hosts by habitat type (Fig. 39).

These calculations assume no differential attraction of snail species to feces bearing the L1's, and that pellet densities were proportional to relative densities of L1's released into the environment by infected hares. Since adult and juvenile hares could have vastly different outputs of L1's (Chap. II), it was also assumed that habitat use was identical for all age groups.

The flow of L1's to snails was higher than the flow of L3's to hares for each species but *V. gouldi* (Fig. 38), indicating that *V. gouldi* had the highest probability of becoming infected. Only a tiny fraction of the *Euconulus fulvus* which might have encountered L1's actually became infected, indicating that this species was an extremely suboptimal host. Input and output flows for *Columella edentula* and *Vertigo ovata* were very low. Both of these pupillid species may have been developmentally acceptable intermediate hosts for *P. boughtoni*, but were unimportant in transmission due to the size and/or dispersion of their populations (Table XIII).

Flow of L1's to each habitat type was roughly proportional to the flow of L3's from each habitat (Fig. 39) except in SS, where high densities of *V. gouldi* increased the flow of L3's, and CUT, where lower *P. boughtoni* abundance in *V. gouldi* decreased the flow of L3's.

The models of parasite flow thus far presented, though simple and based on a limited data set, summarize the probable major factors involved in transmission of *P. boughtoni*. Not investigated were several possible aspects of host and parasite behavior which might facilitate that transmission. For example, some species of snails may be attracted to snowshoe hare fecal pellets as a potential source of food. Emergence of third-stage larvae from snails onto vegetation (Chap. I) could be important. Rowan (1935) reported that *Vertigo elatior* became active in wet weather at temperatures above 4C and climbed a foot or more in height on vegetation, increasing the probability of ingestion by rabbits. *V. gouldi* may behave similarly, but such behavior may not be constant among all snail hosts. *Vallonia pulchella* infected with *P. boughtoni* L3's appeared to exhibit a phototactic response in comparison to uninfected individuals (pers. obs.). This might increase the chance of ingestion by hares. However, there is no report of such parasite-induced alteration of intermediate host behavior for nematodes of the Metastrongyloidea.

The remaining two questions for which flow rates were calculated were: 1) which age class of hare contributed the

greatest flow of first-stage larvae to the system? and, 2) in which month did the greatest flow of first-stage larvae to the environment occur? To answer these questions, it was necessary to calculate the approximate relative numbers of hares in each age group available in each month that transmission might occur. This information was not collected in the present study, so literature sources were used.

Assuming an initial adult cohort of 100 in April (50 male, 50 female), survivors were calculated for each month until October using survival rates for the peak population period of 1970-1971, reported by Keith and Windberg (1978; Table 7). The sizes of the litter groups produced in May, June and July were calculated as the product of the number of female adults, the litter group pregnancy rate for 1970 (Cary and Keith 1979; Table 2), and the mean litter group size for 1970 (Cary and Keith 1979; Table 4). Since differential monthly survival rates for the three litter groups have not been published, they were estimated from available information as follows. The mean six month survival from birth to midwinter for all juvenile snowshoe hares in 1970 at Rochester, Alberta was reported as 0.281 (Keith and Windberg 1978; Table 8). Relative survival of each litter group in increasing populations over approximately the same period of time was 12.2% for the first litter, 11.1% for the second, and 7.1% for the third and fourth (Keith and Windberg 1978; Table 14). The ratio of these percentages is 1:0.910:0.582 and thus:

MEAN SURVIVAL RATE $0.281 = (1x + 0.910x + 0.582x)/3$

$x = 0.338$

Therefore, MEAN MONTHLY SURVIVAL RATES:

LITTER GROUP 1 $= \sqrt[6]{0.388} = 0.835$

LITTER GROUP 2 $= \sqrt[6]{0.910 \times 0.338} = 0.822$

LITTER GROUP 3 $= \sqrt[6]{0.582 \times 0.338} = 0.763$

These survival rates were used to calculate the relative numbers of each litter group available in each month until October.

Calculations of L1 flow from hare age groups and monthly flow of L1's were carried out in a 4 by 7 matrix with age groups assigned to rows and months assigned to columns (Table XIV). In each cell, number of hares was multiplied by the median L1/g of larval output (Chap. II) and the proportion of the mean litter group weight relative to the mean weight of adults for that month. Row products were summed, as were column products. Row sums were divided by the grand sum to produce the relative flow of L1's from each age group. Column sums were divided by the grand sum to produce the relative flow of L1's in each month.

These calculations assume that only first-stage larvae produced in non-winter months play a role in transmission of *P. boughtoni*. They also assume that hare population dynamics at Slave Lake, Alberta in 1981 were similar to those at Rochester, Alberta in 1970, and that the volumes of fecal output from adult and juvenile hares were directly

TABLE XIV. CALCULATIONS OF PROTOSTRONGYLUS BOUGHTONI FLOW FROM EACH HARE AGE GROUP, AND IN EACH SNOW-FREE MONTH, FOR A YEAR OF PEAK HARE POPULATION

AGE GROUP	MONTH							SUM
	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	
Adults								
Number	100	98.90	97.81	96.74	86.97	78.18	70.29	
Ll/g Feces	1.00	0	2.00	0	3.75	0	0.25	
Product	100		195.62		326.14		17.57	639.33
Litter 1								
Number		127.22	106.23	88.70	74.07	61.84	51.64	
Ll/g Feces			0	0	39.80	16.95	0	
Rel. Weight					0.642	0.832		
Product					1892.61	872.09		2764.70
Litter 2								
Number			217.02	178.39	146.64	120.54	99.08	
Ll/g Feces				0	17.65	35.50	5.90	
Rel. Weight					0.428	0.682	0.842	
Product					1107.75	2918.39	492.21	4518.35
Litter 3								
Number				202.69	154.65	118.00	90.03	
Ll/g Feces					0	17.30	63.60	
Rel. Weight						0.470	0.572	
Product						959.46	3275.22	4234.68
SUM	100	0	195.62	0	3326.50	4749.94	3785.00	
GRAND SUM	12157.06							

TABLE XIV. CONTINUED

RELATIVE FLOW OF FIRST-STAGE LARVAE FROM HARE AGE GROUPS		RELATIVE FLOW OF FIRST-STAGE LARVAE FROM ALL HARES BY MONTH	
Adults	0.053	April	0.008
Litter 1	0.227	May	0
Litter 2	0.372	June	0.016
Litter 3	0.348	July	0
		August	0.274
		September	0.391
		October	0.311

proportional to weight. This last assumption was no doubt false, but no data exist that would provide a better estimate.

The calculations show that the different age groups of hares varied in their relative flow rates of *P. boughtoni* L1's. Adults were relatively unimportant in dissemination of L1's in comparison to juveniles. There is some indication (Chap. II) that infrapopulations of *P. boughtoni* in snowshoe hares were regulated by the development of partial immunity; i.e., Bradley's (1974) Type III regulation. Holmes *et al.* (1977) recognized three mechanisms of Type III regulation: 1) modification of the number of parasites which establish or which survive to reproduce; 2) modification of the proportion of surviving parasites which reproduce; and 3) modification of the number of eggs produced per reproducing parasite. None of these mechanisms may be presently discounted.

Regulation of *P. boughtoni* populations within adult hares results in extremely low and often undetectable output of first-stage larvae. Infrapopulations of *P. boughtoni* in juvenile hares increased exponentially for a period of at least two months with proportional increases in the volume of larval output until an immune response halted and reversed the trend. In the peak population year studied, most L1 flow was from the second and third litter groups. Flow from the first group was lower, but still much greater than from the adults. The months of greatest L1 flow from

hares were August, September, and October, with an emphasis on the last two months, coincidental with highest infections in litters 2 and 3.

Relative survival rates of age classes vary throughout the hare population cycle, as do pregnancy rates and litter sizes (Keith and Windberg 1978). Therefore, the relative flow of L1's from each age group may vary over the cycle period due to relative changes in group sizes. Using data for 1974 from the previously mentioned literature sources, and assuming median larval output and relative weights identical to those determined in the present study, relative L1 flow rates were determined for each age group and each month during a population low (Table XV). As in the previous calculations, adults were relatively unimportant in dissemination of L1's. The third litter group, accounting for the second greatest flow for a peak year, accounted for the least proportion in a low year. This was due to a much reduced relative survival rate for the group during the low years. First and second litters accounted for a greater proportion of the flow. This was also evidenced in the relative flow of L1's by month; the greatest flow coincided with greatest infections in those litters.

The periodic fluctuations of snowshoe hare populations have been well documented (Keith and Windberg 1978; Wolff 1980). Concurrent fluctuations in infection with various species of helminth parasites have been reported (Erickson 1944; Cary and Keith 1979). However, Carey and Keith (1979)

TABLE XV. CALCULATIONS OF *PROTOSTRONGYLUS BOUGHTONI* FLOW FROM EACH HARE AGE GROUP, AND IN EACH SNOW-FREE MONTH, FOR A YEAR OF LOW HARE POPULATION

AGE GROUP	MONTH							SUM
	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	
Adults								
Number	100	80.10	64.16	51.39	38.90	29.45	22.29	
L1/g Feces	1.00	0	2.00	0	3.75	0	0.25	
Product	100		128.32		145.88		5.57	379.77
Litter 1								
Number		111.02	81.16	59.32	43.37	31.70	23.17	
L1/g Feces			0	0	39.80	16.95	0	
Rel. Weight					0.642	0.832		
Product					1108.17	447.05		1555.22
Litter 2								
Number			156.51	104.39	69.63	46.44	30.98	
L1/g Feces				0	17.65	35.50	5.90	
Rel. Weight					0.428	0.682	0.842	
Product					526.00	1124.36	153.90	1804.26
Litter 3								
Number				78.63	52.29	34.77	23.12	
L1/g Feces					0	17.30	63.60	
Rel. Weight						0.470	0.572	
Product						282.71	841.09	1123.80
SUM	100	0	128.32	0	1780.05	1854.12	1000.56	
GRAND SUM	4863.05							

TABLE XV. CONTINUED

RELATIVE FLOW OF FIRST-STAGE LARVAE FROM HARE AGE GROUPS		RELATIVE FLOW OF FIRST-STAGE LARVAE FROM ALL HARES BY MONTH	
Adults	0.078	April	0.021
Litter 1	0.320	May	0
Litter 2	0.371	June	0.026
Litter 3	0.231	July	0
		August	0.366
		September	0.381
		October	0.206

determined that prevalence of *P. boughtoni* infection did not vary over the course of the snowshoe hare cycle, remaining at a mean of 43.1%. Whether *P. boughtoni* infrapopulations remain the same size during the hare decline phase is unknown, but such an assumption may not be unreasonable considering the apparently stable rate of transmission.

Immune-mediated, density-dependent parasite population regulation at the individual host level, and a possibly stable transmission rate at widely variant host population densities, are both indicative of extensive coevolution in a host-parasite system (Holmes 1983). As a result of such coevolved mechanisms, the suprapopulation of *P. boughtoni* may not be threatened by extinction during hare population lows, and the hare population may be spared massive mortality due to increased infection rates during population highs. If this is an accurate assessment of the host-parasite system, then mortality due to *P. boughtoni* infection is an unlikely factor in initiation or aggravation of the cyclic decline in snowshoe hare populations. *P. boughtoni* no doubt causes the death of some individual hares in populations, but such mortality would be compensatory (Holmes 1982) rather than additive.

The approach I've taken to describe the transmission of *P. boughtoni* within populations of hosts can be adapted for study of other related metastrongyloids. Seldom in such studies is there time or opportunity to determine the temporal abundance of a parasite in all age groups of a

definitive host. However, the simple models I've employed may be used to identify primary intermediate hosts and likely areas of transmission without this information. Often, fecal samples can be collected from known, individual large ruminants, providing information on relative contribution to parasite flow from various age groups.

If control measures against parasite populations are planned, then knowledge of primary hosts and sites of transmission are essential. Also, some knowledge of the regulatory processes acting on those populations is necessary to avoid misdirected efforts (Bradley 1974). In the present study, regulatory processes were by no means definitively elucidated, but tentative suggestions were advanced which seemed consistent with the data. Extensive experimental research would be necessary to accurately identify the exact mechanisms of population regulation involved.

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APPENDIX I. Histories of snowshoe hares experimentally infected with *Protostrongylus boughtoni*.

NUMBER OF MOTHER	DATE CAPTURED	DATE OF PARTURITION	NUMBERS OF YOUNG	SEX	AGE WHEN EXPOSED TO *P. BOUGHTONI*	AGE WHEN KILLED
S.H. 21	May 27, 1981	June 29, 1981	S.H. 21-1	F	102d	209d
			S.H. 21-2	M	102d	234d
			S.H. 21-3	M	102d	192d
			S.H. 21-4	M	102d	220d
			S.H. 21-5	M	unexposed	95d
S.H. 28	July 14, 1981	July 22, 1981	S.H. 28-1	F	79d	148d
			S.H. 28-2	M	unexposed	79d
			S.H. 28-3	F	79d	124d
			S.H. 28-4	M	79d	96d

APPENDIX II. Number, age and month of acquisition of prenatal and post-partum young examined for transplacental transmission of *Protostrongylus boughtoni*.

FEMALE NUMBER	MONTH YOUNG ACQUIRED	NUMBER OF YOUNG	AGE OF YOUNG
S.H. 2	June 1980	1	1d p.p.
S.H. 2	June 1980	1	2d p.p.
S.H. 8	June 1980	1	11d p.p.
S.H. 8	June 1980	1	12d p.p.
S.H. 8	June 1980	1	21d p.p.
S.H. 22	June 1981	2	stillborn
S.H. 24	June 1981	1	14d p.p.
S.H. 25	June 1981	4	36d p.c.
S.H. 26	June 1981	3	stillborn
S.H. 12	July 1980	1	2d p.p.
S.H. 12	July 1980	1	9d p.p.
S.H. 12	July 1980	3	13d p.p.
S.H. 13	July 1980	3	12d p.p.
S.H. 15	July 1980	1	5d p.p.
S.H. 15	July 1980	2	19d p.p.
S.H. 27	July 1981	1	2d p.p.
S.H. 27	July 1981	1	4d p.p.
S.H. 27	July 1981	1	26d p.p.
S.H. 30	August 1981	1	65d p.p.
S.H. 30	August 1980	1	60d p.p.
S.H. 30	August 1980	1	59d p.p.
S.H. 30	August 1980	1	63d p.p.
A 2	April 1981	2	12d p.c.
A 3	April 1981	2	9d p.c.
A 7	April 1981	3	26d p.c.
A 9	April 1981	3	14d p.c.
A 13	April 1981	2	less than 9d p.c.
A 15	April 1981	2	16d p.c.
A 16	April 1981	2	less than 9d p.c.
A 18	April 1981	1	12d p.c.
A 22	April 1981	2	15d p.c.
A 25	April 1981	1	less than 9d p.c.
M 5	May 1981	6	less than 9d p.c.
M 18	May 1981	3	29d p.c.
M 20	May 1981	4	less than 9d p.c.
M 7	May 1981	3	33d p.c.
J 8	June 1981	4	19d p.c.
J 20	June 1981	4	15d p.c.
J 26	June 1981	4	stillborn
		Total 81	

S.H. numbers were females maintained in the laboratory.
A, M, J, numbers were females trapped and killed on the study area.
p.p. = post-parturition.
p.c. = post-conception (before birth).

APPENDIX III. Quantitative description of each habitat type.

APPENDIX IIIa. VEGETATION VARIABLES DERIVED FROM POINT-CENTERED QUARTER ANALYSIS OF EACH HABITAT TYPE. IMPORTANCE VALUE IS THE SUM OF THE FIRST THREE VARIABLES TO A MAXIMUM OF 300

HABITAT TYPE	TREE SPECIES	RELATIVE DENSITY	RELATIVE DOMINANCE	RELATIVE FREQUENCY	IMPORTANCE VALUE
STAG	*Picea mariana*	95.7	92.6	90.7	279.0
	Pinus contorta	0.5	4.4	1.9	6.8
	Salix spp.	2.0	0.1	3.7	5.8
	Larix laricina	0.8	2.0	2.8	5.6
	Alnus crispa	1.0	0.9	0.9	2.8
SPRUCE SLOPE	*Picea glauca*	53.0	71.9	42.3	167.2
	Abies balsamea	35.8	19.8	38.6	94.2
	Betula papyrifera	6.5	1.1	9.5	17.1
	Populus tremuloides	2.5	5.1	4.2	11.8
	Populus balsamifera	1.5	1.1	3.2	5.8
	Alnus crispa	0.8	0.0	1.6	2.4
	Picea mariana	0.3	0.0	0.5	0.8
MIXED WOOD	*Picea glauca*	24.8	46.1	28.2	99.1
	Populus tremuloides	18.5	29.2	17.1	64.8
	Abies balsamea	28.0	14.4	15.3	57.7
	Betula papyrifera	16.0	5.5	17.1	38.6
	Populus balsamifera	6.8	3.3	8.3	18.4
	Salix spp.	5.8	0.7	6.9	13.4
	Alnus crispa	5.8	0.9	6.0	12.7
	Prunus pensylvanica	0.5	0.2	0.9	1.6
CUT	*Populus balsamifera*	22.5	33.5	20.2	76.2
	Populus tremuloides	22.8	24.9	20.2	67.9
	Betula papyrifera	20.5	14.0	21.0	55.5
	Alnus crispa	13.3	4.1	17.7	35.1
	Salix spp.	13.0	6.4	11.9	31.3
	Picea glauca	6.0	14.8	6.6	28.4
	Pinus contorta	0.5	0.8	0.8	2.1
	Abies balsamea	0.5	0.4	0.4	1.3
	Picea mariana	0.3	0.3	0.4	1.0
	Prunus pensylvanica	0.3	0.1	0.4	0.8
HARD-WOOD	*Populus tremuloides*	45.8	42.2	41.4	129.4
	Populus balsamifera	31.8	43.9	30.5	106.2
	Betula papyrifera	8.5	3.3	8.4	20.2
	Alnus crispa	8.0	0.6	10.3	18.9
	Picea glauca	1.5	8.7	3.0	13.2
	Salix spp.	3.3	1.0	5.4	9.7
	Prunus pensylvanica	0.8	0.1	0.5	1.4
	Picea mariana	0.3	0.0	0.5	0.8

APPENDIX IIIb. PERCENT COVER AND RELATIVE COVER DERIVED FROM LINE-INTERCEPT ANALYSIS OF EACH HABITAT TYPE.

HABITAT TYPE	SHRUB SPECIES	% COVER (RANGE)	RELATIVE COVER
STAG	*Ledum groenlandicum*	42.0(0-78.2)	82.8
	Picea mariana	6.0(0.9-10.4)	11.9
	Lonicera involucrata	1.1(0-5.6)	2.2
	Abies balsamea	0.7(0-3.6)	1.4
	Salix spp.	0.4(0-1.8)	0.8
	Rosa acicularis	0.4(0-1.7)	0.8
	Cornus stolonifera	0.0(0-0.2)	0.1
SPRUCE SLOPE	*Abies balsamea*	14.5(0-43.8)	67.6
	Viburnum trilobum	3.8(0.8-8.0)	17.7
	Lonicera involucrata	2.4(0-7.6)	11.1
	Rosa acicularis	0.7(0-1.5)	3.3
	Cornus stolonifera	0.0(0-0.2)	0.2
	Salix spp.	0.0(0-0.1)	0.1
MIXED WOOD	*Abies balsamea*	14.3(0-42.2)	47.5
	Viburnum trilobum	9.9(0.4-22.9)	32.9
	Lonicera involucrata	2.7(0.5-5.7)	8.9
	Rosa acicularis	1.1(0-4.0)	3.7
	Ledum groenlandicum	0.8(0-4.2)	2.7
	Picea glauca	0.6(0-2.8)	1.9
	Populus balsamifera	0.2(0-0.8)	0.7
	Ribes oxyacanthoides	0.2(0-0.6)	0.7
	Rubus strigosus	0.2(0-0.8)	0.7
	Alnus crispa	0.1(0-0.6)	0.3
CUT	*Lonicera involucrata*	10.7(0.3-23.5)	34.8
	Picea glauca	5.0(0.1-15.2)	16.3
	Viburnum trilobum	4.8(0-15.9)	15.6
	Rosa acicularis	3.8(0-9.1)	12.4
	Rubus strigosus	3.6(0-10.1)	11.7
	Salix spp.	1.8(0-7.0)	5.9
	Alnus crispa	0.3(0-0.6)	0.9
	Abies balsamea	0.2(0-1.0)	0.7
	Populus balsamifera	0.2(0-1.0)	0.7
	Amelanchier alnifolia	0.2(0-0.8)	0.7
	Ribes oxyacanthoides	0.1(0-0.5)	0.3
HARD-WOOD	*Viburnum trilobum*	22.8(9.7-32.1)	51.4
	Lonicera involucrata	13.3(2.2-33.8)	30.0
	Rosa acicularis	5.0(0.5-9.7)	11.2
	Alnus crispa	1.5(0-6.0)	3.4
	Rubus strigosus	0.9(0-4.2)	2.0
	Cornus stolonifera	0.7(0-3.4)	1.6
	Ribes oxyacanthoides	0.1(0-0.7)	0.2
	Abies balsamea	0.0(0-0.3)	0.1
	Populus balsamifera	0.0(0-0.3)	0.1
	Picea glauca	0.0(0-0.1)	0.0

APPENDIX IIIe.

SUMMARY OF VEGETATION VARIABLES DERIVED FROM DAUBENMIRE COVER SCALE ANALYSIS OF EACH HABITAT TYPE. VARIABLES ARE RELATIVE FREQUENCY, RELATIVE COVER, AND IMPORTANCE VALUE WHICH IS THE SUM OF THE FIRST TWO TO A MAXIMUM OF 200

HABITAT TYPE	HERB SPECIES	REL. FREQ.	REL. COV.	IMP. VAL.	HABITAT TYPE	HERB SPECIES	REL. FREQ.	REL. COV.	IMP. VAL.	HABITAT TYPE	HERB SPECIES	REL. FREQ.	REL. COV.	IMP. VAL.
STAG	Bryophyta	19.0	70.0	89.0	SPRUCE	Bryophyta	14.1	57.4	71.5	MIXED	Bryophyta	9.7	33.4	43.1
	Ascomycota	10.3	11.9	22.2	SLOPE	*Cornus canadensis*	11.0	5.6	16.6	WOOD	Graminoae	4.3	12.9	17.2
	Oxycoccus quadripetalus	14.4	4.8	19.2		*Mitella nuda*	8.9	3.4	12.3		*Cornus canadensis*	9.2	6.9	16.1
	Arctostaphylos uva-ursi	8.0	4.3	12.3		*Aralia nudicaulis*	6.4	5.1	11.5		*Mertensia paniculata*	7.2	6.3	13.5
	Cornus canadensis	9.5	1.2	10.7		*Mertensia paniculata*	4.9	5.6	10.5		*Mitella nuda*	8.2	4.8	13.0
	Petasites palmatus	5.3	4.8	10.1		*Linnaea borealis*	7.4	2.3	9.7		*Rubus pubescens*	7.2	4.6	11.8
	Oxycoccus microcarpus	7.6	0.9	8.5		*Maianthemum canadense*	7.1	1.7	8.8		*Aralia nudicaulis*	5.6	5.5	11.1
	Rubus chamaemorus	4.9	1.7	6.6		*Petasites palmatus*	3.7	4.3	8.0		*Petasites palmatus*	7.2	3.4	10.6
	Sullacina trifolia	4.9	1.3	6.2		*Rubus pubescens*	5.2	1.9	7.1		*Linnaea borealis*	5.3	3.9	9.2
	Rosa acicularis	3.8	0.4	4.2		*Rosa acicularis*	5.2	1.2	6.4		*Rosa acicularis*	4.6	3.6	8.2
	Carex spp.	2.3	0.5	2.8		*Lycopodium sp.*	1.2	2.9	4.1		*Equisetum sp.*	4.1	2.1	6.2
	Equisetum sp.	1.9	0.4	2.3		*Viola adunca*	2.8	0.7	3.5		*Maianthemum canadense*	3.1	0.1	3.2
	Lycopodium sp.	1.9	0.3	2.2		*Equisetum sp.*	1.2	1.9	3.1		*Trientalis borealis*	2.4	0.7	3.1
	Rubus pubescens	1.9	0.3	2.2		Graminoae	2.1	0.8	2.9		*Galium boreale*	2.4	0.7	3.1
	Linnaea borealis	1.5	0.2	1.7		Pteridophyta	2.1	0.6	2.7		*Lathyrus venosus*	2.2	0.7	2.9
	Mitella nuda	1.1	0.3	1.4		Ascomycota	2.1	0.6	2.7		*Viola adunca*	1.7	0.9	2.6
	Viola adunca	0.4	0.1	0.5		*Galium triflorum*	1.8	0.7	2.5		*Lycopodium sp.*	1.0	1.3	2.3
	Epilobium angustifolium	0.4	0.1	0.5		*Trientalis borealis*	1.8	0.4	2.2		*Astaea rubra*	1.7	0.6	2.3
	Trientalis borealis	0.4	0.1	0.5		*Astaea rubra*	1.5	0.4	1.9		*Rubus strigosus*	1.4	0.9	2.3
	Galium triflorum	0.4	0.1	0.5		*Viola rugulosa*	1.5	0.4	1.9		*Pyrola asarifolia*	1.7	0.6	2.3
						Galium boreale	1.5	0.4	1.9		*Galium triflorum*	1.7	0.6	2.3
						Pyrola asarifolia	1.5	0.3	1.8		*Epilobium angustifolium*	1.2	0.7	1.9
						Rubus strigosus	1.2	0.3	1.5		*Viola rugulosa*	1.4	0.4	1.8
						Aster sp.	1.2	0.3	1.5		*Rubus chamaemorus*	0.7	1.0	1.7
						Pyrola secunda	0.6	0.1	0.7		Pteridophyta	1.2	0.4	1.6
						Rubus chamaemorus	0.3	0.1	0.4		*Aster sp.*	1.2	0.4	1.6
						Lathyrus venosus	0.3	0.1	0.4		*Oxycoccus quadripetalus*	0.7	0.6	1.3
						Epilobium angustifolium	0.3	0.1	0.4		*Fragaria glauca*	0.5	0.1	0.6
						Mustneon divaricatum	0.3	0.1	0.4		*Pyrola secunda*	0.5	0.1	0.6
						Arctostaphylos uva-ursi	0.3	0.1	0.4		*Carex sp.*	0.2	0.1	0.3
											Ascomycota	0.2	0.1	0.3

APPENDIX IIIc.

CONTINUED

HABITAT TYPE	HERB SPECIES	REL. FREQ.	REL. COV.	IMP. VAL.	HABITAT TYPE	HERB SPECIES	REL. FREQ.	REL. COV.	IMP. VAL.
CUT	Graminese	11.5	26.9	38.4	HARD-WOOD	*Aralia nudicaulis*	6.8	19.1	25.9
	Bryophyta	5.9	32.4	38.3		Graminese	6.8	11.5	18.3
	Equisetum sp.	7.9	9.7	17.6		*Rosa acicularis*	7.9	8.0	15.9
	Mertensia paniculata	8.5	6.1	14.6		*Mertensia paniculata*	6.1	9.5	15.6
	Epilobium angustifolium	8.7	4.2	12.9		*Maianthemum canadense*	5.4	7.3	12.7
	Rosa acicularis	6.7	4.3	11.0		*Rubus pubescens*	6.6	5.6	12.2
	Rubus strigosus	5.4	5.0	10.4		*Epilobium angustifolium*	5.2	5.9	11.1
	Rubus pubescens	5.6	1.6	7.2		*Cornus canadensis*	5.7	4.6	10.3
	Aster sp.	4.6	1.6	6.2		*Mitella nuda*	5.7	4.4	10.1
	Petasites palmatus	3.8	0.9	4.7		*Lathyrus venosus*	3.9	3.0	6.9
	Taraxacum officinale	3.3	0.6	3.9		*Galium boreale*	5.0	1.7	6.7
	Ascomycota	2.8	0.7	3.5		*Equisetum sp.*	2.0	4.0	6.0
	Lathyrus venosus	2.8	0.5	3.3		Bryophyta	3.5	2.4	5.9
	Mainsom divaricatum	2.6	0.7	3.3		*Viola rugulosa*	3.3	2.3	5.6
	Ribes oxyacanthoides	2.3	0.4	2.7		*Pyrola asarifolia*	3.5	2.1	5.6
	Viola americana	2.3	0.4	2.7		*Rubus strigosus*	3.0	1.6	4.6
	Galium boreale	2.3	0.4	2.7		*Linnaea borealis*	2.2	2.2	4.2
	Aralia nudicaulis	1.5	1.1	2.6		*Aster sp.*	2.4	1.7	4.1
	Galium triflorum	2.1	0.3	2.4		*Viola adunca*	2.0	1.3	3.3
	Cornus canadensis	1.5	0.3	1.8		*Galium triflorum*	3.0	0.1	3.1
	Lycopodium sp.	1.3	0.3	1.6		*Actaea rubra*	2.0	0.7	2.7
	Maianthemum canadense	1.3	0.3	1.6		*Petasites palmatus*	1.8	0.6	2.4
	Pyrola asarifolia	1.3	0.3	1.6		*Ribes oxyacanthoides*	1.3	0.6	1.9
	Mitella nuda	1.3	0.2	1.5		*Disporum trachycarpum*	0.7	1.0	1.7
	Fragaria glauca	1.0	0.2	1.2		*Viola americana*	0.9	0.4	1.3
	Carex sp.	0.3	0.5	0.8		Pteridophyta	0.7	0.2	0.9
	Trifolium sp.	0.5	0.1	0.6		*Urtica gracilis*	0.4	0.5	0.9
	Actaea rubra	0.3	0.1	0.4		*Fragaria glauca*	0.7	0.2	0.9
	Pyrola secunda	0.3	0.1	0.4		*Trientalis borealis*	0.7	0.2	0.9
	Solidago sp.	0.3	0.1	0.4		Ascomycota	0.2	0.1	0.3
						Smilacina racemosa	0.2	0.1	0.3
						Pyrola secunda	0.2	0.1	0.3

APPENDIX IV. Number, age group, sex and month of capture of snowshoe hares from the study area examined for *Protostrongylus boughtoni*.

MONTH	ADULTS M	ADULTS F	LITTER 1 M	LITTER 1 F	LITTER 2 M	LITTER 2 F	LITTER 3 M	LITTER 3 F	GROUPED LITTERS M	GROUPED LITTERS F
JAN	7	6								
FEB	7	7								
MAR	6	7								
APR	8	14								
MAY	12	8	0	2						
JUN	15	4	5	5	1	1				
JUL	5	3	5	5	7	5				
AUG	2	6	4	4	5	6	1	0		
SEP	3	4	5	1	5	4	0	2		
OCT	4	2	1	3	4	6	2	1		
NOV	1	5							8	7
DEC	5	2	—	—	—	—	—	—	5	8
TOTAL	75	68	20	20	22	22	3	3	13	15
SUM	261									

M = male
F = female

APPENDIX V. Mean intensities of Protostrongylus boughtoni infection, with standard deviations, for snowshoe hare age group samples from each month.

MONTH	ADULTS		LITTER 1		LITTER 2		LITTER 3		GROUPED LITTERS	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
JAN	12.6	7.5								
FEB	10.2	5.9								
MAR	14.1	7.4								
APR	10.0	8.5								
MAY	6.8	6.8								
JUN	5.5	6.5	1.0	0						
JUL	9.9	6.4	6.7	3.9	4.5	2.6				
AUG	19.3	17.5	145.3	51.3	69.7	49.0	1	0		
SEP	8.4	3.0	48.8	23.6	103.9	73.9	78.5	0.7		
OCT	14.3	9.8	22.8	16.1	56.6	40.6	160.3	108.0		
NOV	14.3	6.6							24.5	19.6
DEC	8.7	5.4							23.4	11.4

$\bar{x}$ = mean
s = standard deviation

APPENDIX VI. *Protostrongylus boughtoni* recovered from snowshoe hares (*Lepus americanus*) of Quebec.

There are no published reports of Protostrongylus boughtoni Goble and Dougherty, 1943 occurring in snowshoe hares (Lepus americanus) of Quebec. Hares from that province were examined to investigate whether a discontinuity in the geographical distribution of the parasite might exist.

Ten snowshoe hares were collected from an area near Saint-Sylvère, Quebec by shooting on December 30 and 31, 1982. Lungs were removed, frozen, and transported to the University of Alberta where they were thawed and examined following the methods outlined in Chapter II. Nematodes recovered were fixed in cold glycerin-alcohol and examined as whole mounts after clearing in lactophenol.

Each of the ten lungs contained adult nematodes identified as P. boughtoni using measurements from the redescription of the species (Goble and Dougherty, 1943, J. Parasitol. 29:397-404). Mean intensity of infection was 16.4 with a range of 4 to 54. Data on ages and sexes of the hosts were not available, but mean intensity did not appear to differ greatly from that of December adults, and grouped litters, in the present study (Appendix V). This report may constitute a range extension for P. boughtoni.